PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 July 2001 (12.07.01)	
International application No. PCT/EP00/09500	**Applicant's or agent's file reference** FB/BC45263
International filing date (day/month/year) 27 September 2000 (27.09.00)	**Priority date** (day/month/year) 30 September 1999 (30.09.99)
Applicant VINALS Y DE BASSOLS, Carlota	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   04 April 2001 (04.04.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Charlotte ENGER

Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US) PCT/EP00/09500

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PRIVETT, Kathryn, Louise
SmithKline Beecham
Corporate Intellectual Property
(CN9.25.1)
980 Great West Road
Brentford
Middlesex TW8 9GS
ROYAUME-UNI

Date of mailing (day/month/year)
11 March 2002 (11.03.02)

Applicant's or agent's file reference
FB/BC45263

IMPORTANT NOTIFICATION

International application No.
PCT/EP00/09500

International filing date (day/month/year)
27 September 2000 (27.09.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
PRIVETT, Kathryn, Louise
SmithKline Beecham
Two New Horizons Court
Brentford
Middlesex TW8 9EP
United Kingdom

State of Nationality:
State of Residence:
Telephone No.: +44 20 8975 2585
Facsimile No.: +44 20 8975 6294
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
PRIVETT, Kathryn, Louise
SmithKline Beecham
Corporate Intellectual Property
(CN9.25.1)
980 Great West Road
Brentford
Middlesex TW8 9GS
United Kingdom

State of Nationality:
State of Residence:
Telephone No.: +44 20 8047 5000
Facsimile No.: +44 20 8047 6894
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Jean-Luc MARTIN

Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US) PCT/EP00/09500

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PRIVETT, Kathryn, Louise
SmithKline Beecham
Two New Horizons Court
Brentford
Middlesex TW8 9EP
ROYAUME-UNI

Date of mailing (day/month/year) 27 November 2001 (27.11.01)	
Applicant's or agent's file reference FB/BC45263	**IMPORTANT NOTIFICATION**
International application No. PCT/EP00/09500	**International filing date** (day/month/year) 27 September 2000 (27.09.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address	State of Nationality	State of Residence
GAULIS, Swann, Roman, Jean-Thomas GlaxoSmithKline Biologicals 89, rue de l'Institut B-1330 Rixensart Belgium	FR	BE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
Additional applicant/inventor for US only.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer François BAECHLER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 004491183

Copy for the Elected Office (EO/US) PCT/EP00/09500

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PRIVETT, Kathryn, Louise
SmithKline Beecham
Two New Horizons Court
Brentford
Middlesex TW8 9EP
ROYAUME-UNI

Date of mailing (day/month/year)
27 November 2001 (27.11.01)

Applicant's or agent's file reference
FB/BC45263

IMPORTANT NOTIFICATION

International application No.
PCT/EP00/09500

International filing date (day/month/year)
27 September 2000 (27.09.00)

1. The following indications appeared on record concerning:
[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:
[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address
CASSART, Jean-Pol
GlaxoSmithKline Biologicals
Rue de l'Institut 89
B-1330 Rixensart
Belgium

State of Nationality: BE | State of Residence: BE

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:
Additional applicant/inventor for US only.

4. A copy of this notification has been sent to:
[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
François BAECHLER

Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US) PCT/EP00/09500

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PRIVETT, Kathryn, Louise
SmithKline Beecham
Two New Horizons Court
Brentford
Middlesex TW8 9EP
ROYAUME-UNI

Date of mailing (day/month/year)
27 November 2001 (27.11.01)

Applicant's or agent's file reference
FB/BC45263

IMPORTANT NOTIFICATION

International application No.
PCT/EP00/09500

International filing date (day/month/year)
27 September 2000 (27.09.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address	State of Nationality	State of Residence
COCHE, Thierry GlaxoSmithKline Biologicals Rue de l'Institut 89 B-1330 Rixensart Belgium	BE	BE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
Additional applicant/inventor for US only.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer François BAECHLER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 004491172

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
SMITHKLINE BEECHAM
Attn. PRIVETT, Kathryn Louise
Two New Horizons Court
Brentford
Middlesex TW8 9EP
UNITED KINGDOM

RECEIVED
26 MAR 2001
NEW HORIZONS COURT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION
(PCT Rule 44.1)

Applicant's or agent's file reference FB/BC45263	Date of mailing *(day/month/year)* 26/03/2001 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/EP 00/ 09500	International filing date *(day/month/year)* 27/09/2000
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because th y are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Pat ntlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 po nl, Fax: (+31-70) 340-3016	Authorized officer Chantal Meyer

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the languag of the international applicati n is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
FB/BC45263		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 09500	27/09/2000	30/09/1999
Applicant		
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   HUMAN TUMOR-ASSOCIATED LAK-4P RELATED POLYNUCLEOTIDES AND POLYPEPTIDES AND THEIR USES

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant. [ ] Non of the figur s.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better charact rizes the invention.

International Application No
PCT/EP 00/09500

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/62 C07K14/47 C07K16/18 C12Q1/68
G01N33/53 A61K38/17 A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 52161 A (INCYTE PHARMACEUTICALS) 8 September 2000 (2000-09-08) abstract page 1, line 1 - line 19 page 2, line 19 -page 5, line 14 page 19, line 5 -page 52, line 10 SEQ ID NOS: 2 and 7 page 53 -page 56; tables 1-4 page 59 -page 61; claims 1-23 SEQ ID NO: 2 page 63 SEQ ID NO: 7 page 66 -page 67 --- -/--	1,5-11, 13-16, 22,23, 27-32, 34-37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 March 2001	26/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuchs, U

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 35937 A (HUMAN GENOME SCIENCES, INC.) 22 June 2000 (2000-06-22) abstract page 2, line 13 - line 21 gene no.: 22 page 59, line 17 -page 61, line 12 page 118; table 1 page 122, line 1 -page 238, line 14 page 241, line 25 -page 249, line 6 page 263, line 21 -page 265, line 23 page 279, line 6 -page 294, line 24 page 296, line 25 -page 307, line 26 page 309; table 2 page 432 -page 437; claims 1-23 SEQ ID NO: 32 page 455 -page 456	5-8, 13-17, 22,23, 27-29, 32,34,36

A	EMBL database, Heidelberg, FRG Emhum1 accession number AB002405 9 January 1998 ABE, Y. & TAKAOKA, Y.: "Homo sapiens mRNA for LAK-4p, complete cds." XP002162738 the whole document	1-25, 27-37
A	-& EMBL database, Heidelberg, FRG Trembl accession number O43284 1 June 1998 ABE, Y. & TAKAOKA, Y.: "LAK-4P, Homo sapiens" XP002162739 the whole document	1-25, 27-37

A	EMBL database, Heidelberg, FRG Emest_Hum7 accession number AI799626 7 July 1999 NCI-CGAP: "to74b03.x1 NCI_CGAP_Gas4 Homo sapiens cDNA clone IMAGE: 2183981 3' similar to TR: O43284 O43284 LAK-4P, mRNA sequence" XP002162740 cited in the application the whole document	1-25, 27-37

A	EMBL database, Heidelberg, FRG Emest_Hum8 accession number AI921465 2 August 1999 NCI-CGAP: "wo25d05.x1 NCI_CGAP_Gas4 Homo sapiens cDNA clone IMAGE: 2456361 3', mRNA sequence" XP002162741 the whole document	1-25, 27-37

	-/--	

2

International Application No
PCT/EP 00/09500

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EMBL database, Heidelberg, FRG Emest_Hum5 accession number AI346622 7 January 1999 NCI-CGAP: "qp46f08.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE: 1926087 3', mRNA sequence" XP002162742 cited in the application the whole document ---	1-25, 27-37
A	EMBL database, Heidelberg, FRG Emest_Hum25 accession number AA172244 24 December 1996 HILLIER, L. ET AL.: "zo96g02.r1 Stratagene ovarian cancer (#937219) Homo sapiens cDNA clone IMAGE: 594770 5', mRNA sequence" XP002162743 cited in the application the whole document ---	1-25, 27-37
A	EMBL database, Heidelberg, FRG Emest_Hum7 accession number AI697014 4 June 1999 NCI-CGAP: "wc76h09.x1 NCI_CGAP_Pan1 Homo sapiens cDNA clone IMAGE: 2324609 3', mRNA sequence" XP002162744 cited in the application the whole document -----	1-25, 27-37

2

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 26 completely and 27 partially

Claims 26 completely and 27 partially refer to an antagonist to the polypeptide of claims 1 to 5 without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT).

No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the result ot be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNA :AL SEARCH REPORT

International application No.
PCT/EP 00/09500

Box I Observations wher certain claims were found unsearchable (Continuati n of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 24 and 25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 26 completely and 27 partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search f es were accompanied by the applicant's protest.

[ ] No protest accompani d the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/09500

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0052161 A	08-09-2000	AU 3506800 A	21-09-2000
WO 0035937 A	22-06-2000	AU 3124000 A	03-07-2000

by fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DALTON, Marcus Jonathan William
GLAXOSMITHKLINE
Corporate Intellectual Property
Two New Horizons Court
Brentford, Middlesex TW8 9EP
GRANDE BRETAGNE

FAX: 0044 20 8975 6294

GlaxoSmithKline
Corporate IP
Received BRENTFORD
1 - FEB 2002
ATTY: ADMIN:
IPM : N/A | ON | UPDATED ON:
ATTY CHECKED/FILE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 25.01.2002
Applicant's or agent's file reference MJWD/BC45263	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/09500	27/09/2000	30/09/1999

Applicant
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of th IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	MYLONAS, E Tel.+49 89 2399-7746

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
MJWD/BC45263		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/09500	27/09/2000	30/09/1999
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/04/2001	25.01.2002
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Friedrich, C Telephone No. +49 89 2399 7721

Form PCT/IPEA/409 (cover sheet) (January 1994)

I. Basis of th report

1. With regard to the **elem nts** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-72 as originally filed

Claims, No.:

1-37 as received on 20/11/2001 with letter of 19/11/2001

Drawings, sheets:

1/2,2/2 as originally filed

Sequence listing part of the description, pages:

63-72, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 24-27.

because:

   ☒ the said international application, or the said claims Nos. 24-25 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 26-27.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Articl 35(2) with r gard to n velty, inventiv step or industrial applicability; citations and explanations supp rting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7, 9-12, 14-25, 28-37
	No:	Claims	8, 13
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7, 9-12, 14-25, 28-37
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	1-7, 9-12, 14-23, 28-37

2. Citations and explanations
see separate sheet

Reference is made to the following documents:

D1: EMBL database, Heidelberg, FRG accession number AI799626, 7 Jul. 1999

D2: EMBL database, Heidelberg, FRG accession number AI921465, 2 Aug. 1999

D3: EMBL database, Heidelberg, FRG accession number AI346622, 7 Jan. 1999

D4: EMBL database, Heidelberg, FRG accession number AA172244, 24 Dec. 1996

D5: EMBL database, Heidelberg, FRG accession number AI697014, 4 Jun. 1999

Introduction

The present application concerns amino acid and DNA sequences based on SEQ ID No: 1-5 and 7, said DNA sequences transcribed at higher levels in colon tumour tissue compared to normal tissue (claims 1-13 and 32). It furthermore relates to recombinant expression of the respective protein (claims 14-16), peptide and DNA vaccines (claims 17-21, and 33), antibodies directed against the polypeptides (claim 22), a method to identify agonists and antagonists (claim 23), methods of treatment of related conditions (claims 24-25), diagnostic methods (claims 28-31), and the use of polypeptides and polynucleotides for the treatment of carcinoma (claims 34-37).

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 24 and 25 relate to subject-matter considered by this authority to be covered by the provisions of Rule 67.1(iv), PCT (the treatment of the human or animal body). Consequently, no opinion will be formulated with regard to industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i), PCT.

Re Item V

Reasoned statement under Art.35(2), PCT with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty, Art.33(1) and (2), PCT

Claims 8 and 13 concern, among others, polynucleotides encoding immunogenic fragments of polypeptides according to SEQ ID No: 2 or 4 (immunogenic fragments according to the definition on page 2 typically will comprise 7, preferably 9 or 10 contiguous amino acids from SEQ ID NO:2 or 4). D1-D5 disclose DNA sequences encoding such polypeptide fragments and thus appear to be detrimental for novelty of

subject-matter referred to in said claims (see Art. 33 (2), PCT).

Since DNA and amino acid sequences according to SEQ ID No.1-5 and 7 have not been disclosed in the prior art subject-matter referred to in claims 1-7, 9-12, 14-25, and 28-37 appears to be novel under Art. 33 (2), PCT.

2. Inventive Step, Art.33(1) and (3), PCT

Subject-matter of claims 1-7, 9-12, 14-25, and 28-37 concerns amino acid and DNA sequences according to SEQ ID No: 1-5 and 7, their potential use in the preparation of vaccines and antibodies, methods to identify agonists and antagonists, diagnostic methods, and methods of treatment, all based on standard techniques well known and routine to those of skill in the art. The functions of said sequences as provided in the present specification are purely hypothetical. For example the polypeptides are believed to be important immunogens for specific prophylactic or therapeutic immunization against tumours that can also be used to diagnose the occurrence of tumour cells (page 1 of the specification). This, however, is only credible if it is experimentally confirmed since a colon cancer-specific gene must not necessarily be suitable for therapy or diagnostics (see also Rule 5.1(a)(iii), PCT). Attention is drawn to Fig.1 and Fig.2 of the specification showing that the gene is in fact not specifically expressed in colon cancer (see fallopian tube, ileon, lung, rectum, small intestine, spleen, stomach, trachea, and prostate expression and Fig.2) and does not unequivocally distinguish tumorous from non-tumorous tissue (Fig.1). Furthermore colon cancer specific DNA and amino acid sequences were part of the prior art at the filing date of the present application (see e.g. D3 or page 54 of the present specification). Any one of them may serve as the closest prior art (see also Rule 64, PCT). Consequently the underlying technical problem can only be considered as the provision of further DNA sequences specifically expressed (at best) in colon cancer and not the provision of nucleotides and amino acids for the treatment or diagnosis of cancer, as specified in the present application on pages 1, 20 and 35. Due to the lack of provision of a biological function any new colon cancer specific DNA or polypeptide sequence qualifies as a solution to said problem. Since the provision of differentially expressed DNA sequences requires not more than routine experimentation (cDNA subtraction libraries) the solution put forward by the present application amounts to not more than an arbitrary selection which is not justified by any unexpected technical effect caused by the technical features which distinguish said claimed sequences from

any other sequences. Thus subject-matter of claims 1-7, 9-12, 14-25, and 28-37 does not appear to be inventive according to Art.33(1) and (3), PCT.

3. Industrial Applicability, Art.33 (1) and (4) and Rule 5.1(vi), PCT

Since the present application only discloses hypothetical functions of the claimed amino acid and DNA sequences (diagnosis and therapy of colon cancer) which are not experimentally confirmed the potential fields of use in industry or research (prevention, treatment or diagnosis of disease) for said sequences are totally speculative. The generation of amino acid and DNA sequences without any purpose is not industrially meaningful according to Rule 5.1(vi), PCT, which requires that, when it is not obvious from the nature of the invention, the way in which an invention is capable of exploitation in industry be indicated. The present specification does not fulfill this requirement and therefore subject-matter referred to in claims 1-7, 9-11, 14-23, and 28-37 does not appear to be industrially applicable under Art.33(1) and (4), PCT.

--/--

BC45263.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23417 A3

(51) International Patent Classification[7]: C12N 15/12, 15/62, C07K 14/47, 16/18, C12Q 1/68, G01N 33/53, A61K 38/17, 39/00

(21) International Application Number: PCT/EP00/09500

(22) International Filing Date:
27 September 2000 (27.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9923154.0 30 September 1999 (30.09.1999) GB
0016839.3 7 July 2000 (07.07.2000) GB

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: VINALS Y DE BASSOLS, Carlota [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).

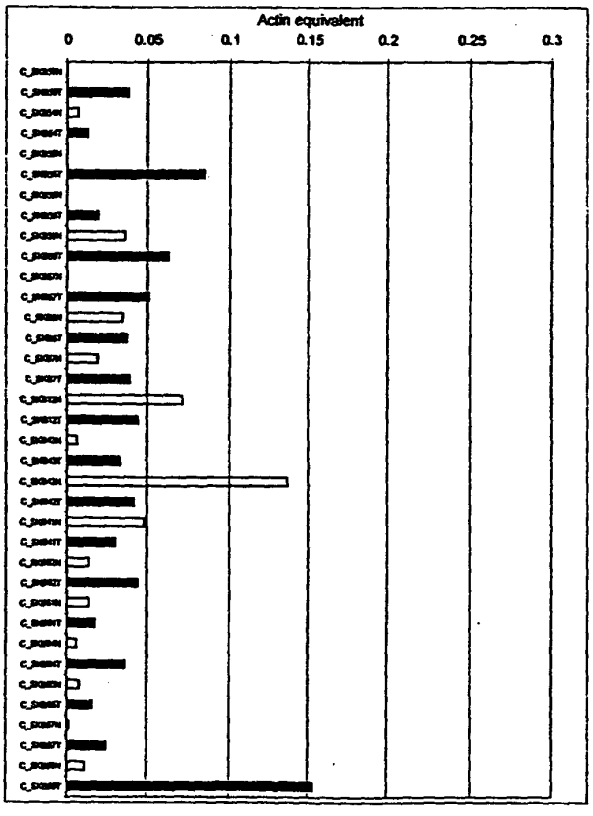
(74) Agent: PRIVETT, Kathryn, Louise; SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian

[Continued on next page]

(54) Title: HUMAN TUMOR-ASSOCIATED LAK-4P RELATED POLYNUCLEOTIDES AND POLYPEPTIDES AND THEIR USES



(57) Abstract: CASB6411 polypeptides and polynucleotides and methods for producing such polpeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing CASB6411 polypeptides and polynucleotides in diagnostics, and vaccines for prophylactic and therapeutic treatment of cancers, particularly ovarian and colon cancers, autoimmune diseases, and related conditions.

WO 01/23417 A3

patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *With international search report.*

— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

(88) Date of publication f the internati nal search report:
17 May 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Int. ..tional Application No
T/EP 00/09500

A. CLASSIFICATION OF SUBJECT M... A
IPC 7 C12N15/12 C12N15/62 C07K14/47 C07K16/18 C12Q1/68
G01N33/53 A61K38/17 A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 52161 A (INCYTE PHARMACEUTICALS) 8 September 2000 (2000-09-08) abstract page 1, line 1 - line 19 page 2, line 19 -page 5, line 14 page 19, line 5 -page 52, line 10 SEQ ID NOS: 2 and 7 page 53 -page 56; tables 1-4 page 59 -page 61; claims 1-23 SEQ ID NO: 2 page 63 SEQ ID NO: 7 page 66 -page 67 --- -/--	1,5-11, 13-16, 22,23, 27-32, 34-37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 March 2001	26/03/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuchs, U

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/EP 00/09500

C.(Continuation) DOCUMENTS CONS. ED TO BE RELEVANT

Category ˇ	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 35937 A (HUMAN GENOME SCIENCES, INC.) 22 June 2000 (2000-06-22) abstract page 2, line 13 - line 21 gene no.: 22 page 59, line 17 -page 61, line 12 page 118; table 1 page 122, line 1 -page 238, line 14 page 241, line 25 -page 249, line 6 page 263, line 21 -page 265, line 23 page 279, line 6 -page 294, line 24 page 296, line 25 -page 307, line 26 page 309; table 2 page 432 -page 437; claims 1-23 SEQ ID NO: 32 page 455 -page 456	5-8, 13-17, 22,23, 27-29, 32,34,36
A	EMBL database, Heidelberg, FRG Emhum1 accession number AB002405 9 January 1998 ABE, Y. & TAKAOKA, Y.: "Homo sapiens mRNA for LAK-4p, complete cds." XP002162738 the whole document	1-25, 27-37
A	-& EMBL database, Heidelberg, FRG Trembl accession number O43284 1 June 1998 ABE, Y. & TAKAOKA, Y.: "LAK-4P, Homo sapiens" XP002162739 the whole document	1-25, 27-37
A	EMBL database, Heidelberg, FRG Emest_Hum7 accession number AI799626 7 July 1999 NCI-CGAP: "to74b03.x1 NCI_CGAP_Gas4 Homo sapiens cDNA clone IMAGE: 2183981 3' similar to TR: O43284 O43284 LAK-4P, mRNA sequence" XP002162740 cited in the application the whole document	1-25, 27-37
A	EMBL database, Heidelberg, FRG Emest_Hum8 accession number AI921465 2 August 1999 NCI-CGAP: "wo25d05.x1 NCI_CGAP_Gas4 Homo sapiens cDNA clone IMAGE: 2456361 3', mRNA sequence" XP002162741 the whole document	1-25, 27-37

-/—

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ..tional Application No
T/EP 00/09500

C.(Continuation) DOCUMENTS CONSI.. ..D TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EMBL database, Heidelberg, FRG Emest_Hum5 accession number AI346622 7 January 1999 NCI-CGAP: "qp46f08.x1 NCI_CGAP_Co8 Homo sapiens cDNA clone IMAGE: 1926087 3', mRNA sequence" XP002162742 cited in the application the whole document	1-25, 27-37
A	EMBL database, Heidelberg, FRG Emest_Hum25 accession number AA172244 24 December 1996 HILLIER, L. ET AL.: "zo96g02.r1 Stratagene ovarian cancer (#937219) Homo sapiens cDNA clone IMAGE: 594770 5', mRNA sequence" XP002162743 cited in the application the whole document	1-25, 27-37
A	EMBL database, Heidelberg, FRG Emest_Hum7 accession number AI697014 4 June 1999 NCI-CGAP: "wc76h09.x1 NCI_CGAP_Pan1 Homo sapiens cDNA clone IMAGE: 2324609 3', mRNA sequence" XP002162744 cited in the application the whole document	1-25, 27-37

2

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 26 completely and 27 partially

Claims 26 completely and 27 partially refer to an antagonist to the polypeptide of claims 1 to 5 without giving a true technical characterization. Moreover, no such compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT).

No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the result ot be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ational Application No
T/EP 00/09500

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0052161 A	08-09-2000	AU 3506800 A	21-09-2000
WO 0035937 A	22-06-2000	AU 3124000 A	03-07-2000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23417 A2

(51) International Patent Classification[7]: C07K 14/00

(21) International Application Number: PCT/EP00/09500

(22) International Filing Date:
27 September 2000 (27.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9923154.0 30 September 1999 (30.09.1999) GB
0016839.3 7 July 2000 (07.07.2000) GB

(71) Applicant *(for all designated States except US)*: **SMITHKLINE BEECHAM BIOLOGICALS S.A.** [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **VINALS Y DE BASSOLS, Carlota** [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE).

(74) Agent: **PRIVETT, Kathryn, Louise**; SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/23417 A2

(54) Title: NOVEL COMPOUNDS

(57) Abstract: CASB6411 polypeptides and polynucleotides and methods for producing such polpeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing CASB6411 polypeptides and polynucleotides in diagnostics, and vaccines for prophylactic and therapeutic treatment of cancers, particularly ovarian and colon cancers, autoimmune diseases, and related conditions.

Novel Compounds

The present invention relates to polynucleotides, herein referred to as CASB6411 polynucleotides, polypeptides encoded thereby (referred to herein as CASB6411 polypeptides), recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including the treatment of cancer and autoimmune diseases and other related conditions. In a further aspect, the invention relates to methods for identifying agonists and antagonists/inhibitors using the materials provided by the invention, and treating conditions associated with CASB6411 polypeptide imbalance with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate CASB6411 polypeptide activity or levels.

Polypeptides and polynucleotides of the present invention are believed to be important immunogens for specific prophylactic or therapeutic immunization against tumours, because they are specifically expressed or highly over-expressed in tumours compared to normal cells and can thus be targeted by antigen-specific immune mechanisms leading to the destruction of the tumour cell. They can also be used to diagnose the occurrence of tumour cells. Furthermore, their inappropriate expression in certain circumstances can cause an induction of autoimmune, inappropriate immune responses, which could be corrected through appropriate vaccination using the same polypeptides or polynucleotides. In this respect the most important biological activities to our purpose are the antigenic and immunogenic activities of the polypeptide of the present invention. A polypeptide of the present invention may also exhibit at least one other biological activity of a CASB6411 polypeptide, which could qualify it as a target for therapeutic or prophylactic intervention different from that linked to the immune response.

In a first aspect, the present invention relates to CASB6411 polypeptides. Such peptides include isolated polypeptides comprising an amino acid sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 OR 4 over the entire length of SEQ ID NO:2 OR 4. Such polypeptides include those comprising the amino acid of SEQ ID NO:2 OR 4.

Further peptides of the present invention include isolated polypeptides in which the amino acid sequence has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to the amino acid sequence of SEQ ID NO:2 OR 4 over the entire length of SEQ ID NO:2 OR 4. Such polypeptides include the polypeptide of SEQ ID NO:2 OR 4.

Further peptides of the present invention include isolated polypeptides encoded by a polynucleotide comprising the sequence contained in SEQ ID NO:1.

The invention also provides an immunogenic fragment of a CASB6411 polypeptide, that is a contiguous portion of the CASB6411 polypeptide which has the same or similar immunogenic properties to the polypeptide comprising the amino acid seqeunce of SEQ ID NO:2 OR 4. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the CASB6411 polypeptide. Such an immunogenic fragment may include, for example, the CASB6411 polypeptide lacking an N-terminal leader sequence, a transmembrane domain or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of CASB6411 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 OR 4 over the entire length of SEQ ID NO:2 OR 4. Preferably an immunogenic fragment according to the invention comprises at least one epitope.

Peptide fragments incorporating an epitope of CASB6411 typically will comprise at least 7, preferably 9 or 10 contiguous amino acids from SEQ ID NO:2 OR 4. Preferred epitopes are shown in SEQ ID NO:9 to SEQ ID NO:72.

Peptides that incorporate these epitopes form a preferred aspect of the present invention. Mimotopes which have the same characteristics as these epitopes, and immunogens comprising such mimotopes which generate an immune response which cross-react with

an epitope in the context of the CASB6411 molecule, also form part of the present invention.

The present invention, therefore, includes isolated peptides encompassing these epitopes themselves, and any mimotope thereof. The meaning of mimotope is defined as an entity which is sufficiently similar to the native CASB6411 epitope so as to be capable of being recognised by antibodies which recognise the native molecule; (Gheysen, H.M., et al., 1986, Synthetic peptides as antigens. Wiley, Chichester, Ciba foundation symposium 119, p130-149; Gheysen, H.M., 1986, Molecular Immunology, 23,7, 709-715); or are capable of raising antibodies, when coupled to a suitable carrier, which antibodies cross-react with the native molecule.

Peptide mimotopes of the above-identified epitopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides of the present invention may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine to the epitope. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. This reduces the conformational degrees of freedom of the peptide, and thus increases the probability that the peptide is presented in a conformation which most closely resembles that of the peptide as found in the context of the whole molecule. For example, the peptides may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide. Those skilled in the art will realise that such modified peptides, or mimotopes, could be a wholly or partly non-peptide mimotope wherein the constituent residues are not necessarily confined to the 20 naturally occurring amino acids. In addition, these may be cyclised by techniques known in the art to constrain the peptide into a conformation that closely resembles its shape when the peptide sequence is in the context of the whole molecule. A preferred method of cyclising a peptide comprises the addition of a pair of cysteine residues to allow the formation of a disulphide bridge.

Further, those skilled in the art will realise that mimotopes or immunogens of the present invention may be larger than the above-identified epitopes, and as such may comprise the sequences disclosed herein. Accordingly, the mimotopes of the present invention may consist of addition of N and/or C terminal extensions of a number of other natural residues at one or both ends. The peptide mimotopes may also be retro sequences of the natural sequences, in that the sequence orientation is reversed; or alternatively the sequences may be entirely or at least in part comprised of D-stereo isomer amino acids (inverso sequences). Also, the peptide sequences may be retro-inverso in character, in that the sequence orientation is reversed and the amino acids are of the D-stereoisomer form. Such retro or retro-inverso peptides have the advantage of being non-self, and as such may overcome problems of self-tolerance in the immune system.

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the epitopes of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native peptide. This approach may have significant advantages by allowing the possibility of identifying a peptide with enhanced immunogenic properties, or may overcome any potential self-antigen tolerance problems which may be associated with the use of the native peptide sequence. Additionally this technique allows the identification of a recognition pattern for each native-peptide in terms of its shared chemical properties amongst recognised mimotope sequences.

The covalent coupling of the peptide to the immunogenic carrier can be carried out in a manner well known in the art. Thus, for example, for direct covalent coupling it is possible to utilise a carbodiimide, glutaraldehyde or (N-[γ-maleimidobutyryloxy] succinimide ester, utilising common commercially available heterobifunctional linkers such as CDAP and SPDP (using manufacturers instructions). After the coupling reaction, the immunogen can easily be isolated and purified by means of a dialysis method, a gel filtration method, a fractionation method etc.

The types of carriers used in the immunogens of the present invention will be readily known to the man skilled in the art. The function of the carrier is to provide cytokine help in order to help induce an immune response against the peptide. A non-exhaustive list of carriers which may be used in the present invention include: Keyhole limpet Haemocyanin (KLH), serum albumins such as bovine serum albumin (BSA), inactivated bacterial toxins such as tetanus or diptheria toxins (TT and DT), or recombinant fragments thereof (for example, Domain 1 of Fragment C of TT, or the translocation domain of DT), or the purified protein derivative of tuberculin (PPD). Alternatively the mimotopes or epitopes may be directly conjugated to liposome carriers, which may additionally comprise immunogens capable of providing T-cell help. Preferably the ratio of mimotopes to carrier is in the order of 1:1 to 20:1, and preferably each carrier should carry between 3-15 peptides.

In an embodiment of the invention a preferred carrier is Protein D from *Haemophilus influenzae* (EP 0 594 610 B1). Protein D is an IgD–binding protein from *Haemophilus influenzae* and has been patented by Forsgren (WO 91/18926, granted EP 0 594 610 B1). In some circumstances, for example in recombinant immunogen expression systems it may be desirable to use fragments of protein D, for example Protein D 1/3rd (comprising the N-terminal 100-110 amino acids of protein D (GB 9717953.5)).

Another preferred method of presenting the peptides of the present invention is in the context of a recombinant fusion molecule. For example, EP 0 421 635 B describes the use of chimaeric hepadnavirus core antigen particles to present foreign peptide sequences in a virus-like particle. As such, immunogens of the present invention may comprise peptides presented in chimaeric particles consisting of hepatitis B core antigen. Additionally, the recombinant fusion proteins may comprise the mimotopes of the present invention and a carrier protein, such as NS1 of the influenza virus. For any recombinantly expressed protein which forms part of the present invention, the nucleic acid which encodes said immunogen also forms an aspect of the present invention.

Peptides used in the present invention can be readily synthesised by solid phase procedures well known in the art. Suitable syntheses may be performed by utilising "T-boc" or "F-moc" procedures. Cyclic peptides can be synthesised by the solid phase

procedure employing the well-known "F-moc" procedure and polyamide resin in the fully automated apparatus. Alternatively, those skilled in the art will know the necessary laboratory procedures to perform the process manually. Techniques and procedures for solid phase synthesis are described in 'Solid Phase Peptide Synthesis: A Practical Approach' by E. Atherton and R.C. Sheppard, published by IRL at Oxford University Press (1989). Alternatively, the peptides may be produced by recombinant methods, including expressing nucleic acid molecules encoding the mimotopes in a bacterial or mammalian cell line, followed by purification of the expressed mimotope. Techniques for recombinant expression of peptides and proteins are known in the art, and are described in Maniatis, T., Fritsch, E.F. and Sambrook et al., *Molecular cloning, a laboratory manual*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

The polypeptides or immunogenic fragment of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins.

Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

Fusion partners include protein D from *Haemophilus influenza* B and the non-structural protein from influenzae virus, NS1 (hemagglutinin). Another immunological fusion partner is the protein known as LYTA. Preferably the C terminal portion of the molecule is used. Lyta is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase, amidase LYTA, (coded by the lytA gene {Gene, 43 (1986) page 265-272} an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of E.coli C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat portion of the Lyta molecule found in the C terminal end starting at residue 178, for example residues 188 - 305.

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

In a further aspect, the present invention relates to CASB6411 polynucleotides. Such polynucleotides include isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to the amino acid sequence of SEQ ID NO:2 OR 4, over the entire length of SEQ ID NO:2 OR 4. In this regard, polypeptides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identity are more highly preferred, and those with at least 99% identity are most highly preferred. Such polynucleotides include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 or 3 encoding the polypeptide of SEQ ID NO:2 OR 4 respectively.

Further polynucleotides of the present invention include isolated polynucleotides comprising a nucleotide sequence that has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2 OR 4, over the entire coding region. In this regard, polynucleotides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identity are more highly preferred, and those with at least 99% identity are most highly preferred.

Further polynucleotides of the present invention include isolated polynucleotides comprising a nucleotide sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to SEQ ID NO:1 or 3 or to the coding sequence of SEQ ID NO:1 or 3 over the entire length of SEQ ID NO:1 or 3 or over the entire length of the coding sequence of SEQ ID NO:1 or 3 respectively. In this regard, polynucleotides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identiy are more highly preferred, and those with at least 99% identity are most highly preferred. Such polynucleotides include a

polynucleotide comprising the polynucleotide of SEQ ID NO:1 or 3 as well as the polynucleotide of SEQ ID NO:1 or 3 or the coding region of SEQ ID NO:1 or 3. Said polynucleotide can be inserted in a suitable plasmid or recombinant microrganism vector and used for immunization (see for example Wolff et. al., Science 247:1465-1468 (1990); Corr et. al., J. Exp. Med. 184:1555-1560 (1996); Doe et. al., Proc. Natl. Acad. Sci. 93:8578-8583 (1996)).

The invention also provides polynucleotides which are complementary to all the above described polynucleotides.

The invention also provides a fragment of a CASB6411 polynucleotide which when administered to a subject has the same immunogenic properties as the polynucleotide of SEQ ID NO:1 or 3.

The invention also provides a polynucleotide encoding an immunological fragment of a CASB6411 polypeptide as hereinbefore defined.

The fragments have a level of immunogenic activity of at least about 50%, preferably at least about 70% and more preferably at least about 90% of the level of immunogenic activity of a polypeptide sequence set forth in SEQ ID NO:2 OR 4 or a polypeptide sequence encoded by a polynucleotide sequence set forth in SEQ ID NO: 1.

The polypeptide fragments according to the invention preferably comprise at least about 5, 10, 15, 20, 25, 50, or 100 contiguous amino acids, or more, including all intermediate lengths, of a polypeptide composition set forth herein, such as those set forth in SEQ ID NO:2 OR 4, or those encoded by a polynucleotide sequence set forth in a sequence of SEQ ID NO:1.

The nucleotide sequence of SEQ ID NO:1 is a cDNA sequence which comprises a polypeptide encoding sequence (nucleotide 349 to 1761) encoding a polypeptide of 460 amino acids, the polypeptide of SEQ ID NO:2. The nucleotide sequence encoding the polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:1 or it may be a sequence other than the one contained in SEQ

ID NO:1, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2. The polypeptide of the SEQ ID NO:2 is structurally related to other proteins of the LAK-4p family, having homology and/or structural similarity with Homo sapiens LAK-4p (GenBank accession BAA24179).

SEQ ID NO:3 is a cDNA sequence which is an alternative isoform of CASB6411 SEQ ID NO:1, and probably the result of alternative exon splicing. SEQ ID NO:3 comprises a polypeptide encoding sequence (nucleotide 382 to 844) encoding a polypeptide of 154 aminoacids, the polypeptide of SEQ ID NO:4. SEQ ID NO:4 is a truncated form of SEQ ID NO:2 polypeptide. The nucleotide sequence encoding the polypeptide of SEQ ID NO:4 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:3 or it may be a sequence other than the one contained in SEQ ID NO:3, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:4.

Preferred polypeptides and polynucleotides of the present invention are expected to have, *inter alia*, similar biological functions/properties to their homologous polypeptides and polynucleotides. Furthermore, preferred polypeptides, immunological fragments and polynucleotides of the present invention have at least one activity of either SEQ ID NO:1 or 3 or SEQ ID NO:2 OR 4, as appropriate.

The present invention also relates to partial or other incomplete polynucleotide and polypeptide sequences which were first identified prior to the determination of the corresponding full length sequences of SEQ ID NO:1 or 3 and SEQ ID NO:2 OR 4.

Accordingly, in a further aspect, the present invention provides for an isolated polynucleotide which:

(a) comprises a nucleotide sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% identity to SEQ ID NO:5 and 7 over the entire length of SEQ ID NO:5 and 7, respectively;

(b) has a nucleotide sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity,

even more preferably at least 97-99% identity, to SEQ ID NO:1 or 3 over the entire length of SEQ ID NO:5 and 7;

(c) the polynucleotide of SEQ ID NO:5 and 7; or

(d) a nucleotide sequence encoding a polypeptide which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% identity, to the amino acid sequence of SEQ ID NO:6 and 8, over the entire length of SEQ ID NO:6 and 8 , respectively,;

as well as the polynucleotide of SEQ ID NO:5 and 7.

The present invention further provides for a polypeptide which:

(a) comprises an amino acid sequence which has at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:6 OR 8 over the entire length of SEQ ID NO:6 and 8;

(b) has an amino acid sequence which is at least 70% identity, preferably at least 80% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to the amino acid sequence of SEQ ID NO:6 OR 8 over the entire length of SEQ ID NO:6 and 8;

(c) comprises the amino acid of SEQ ID NO:6 and 8; and

(d) is the polypeptide of SEQ ID NO:6 and 8;

as well as polypeptides encoded by a polynucleotide comprising the sequence contained in SEQ ID NO:5 and 7.

Polynucleotides of the present invention may be obtained, using standard cloning and screening techniques, from a cDNA library derived from mRNA in cells of human, colorectal tumours, stomach tumors and normal stomach, normal pancreas and pancreas tumors, ovarian tumors, lung tumors, and normal prostate, (for example Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring harbor Laboratory Press, Cold Spring harbor, N.Y. (1989)). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

When polynucleotides of the present invention are used for the recombinant production of polypeptides of the present invention, the polynucleotide may include the coding sequence for the mature polypeptide, by itself; or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, Proc Natl Acad Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further embodiments of the present invention include polynucleotides encoding polypeptide variants which comprise the amino acid sequence of SEQ ID NO:2 OR 4 and in which several, for instance from 5 to 10, 1 to 5, 1 to 3, 1 to 2 or 1, amino acid residues are substituted, deleted or added, in any combination.

Polynucleotides which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or 3, may be used as hybridization probes for cDNA and genomic DNA or as primers for a nucleic acid amplification (PCR) reaction, to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes (including genes encoding paralogs from human sources and orthologs and paralogs from species other than human) that have a high sequence similarity to SEQ ID NO:1 or 3. Typically these nucleotide sequences are 70% identical, preferably 80% identical, more preferably 90% identical, most preferably 95% identical to that of the referent. The probes or primers will generally comprise at least 15 nucleotides, preferably, at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will have between 30 and 50 nucleotides. Particularly preferred primers will have between 20 and 25 nucleotides. In particular, polypeptides or polynucleotides derived from sequences from homologous animal origin could be used as immunogens to obtain a cross-reactive immune response to the human gene.

A polynucleotide encoding a polypeptide of the present invention, including homologs from species other than human, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or 3 or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan. Preferred stringent hybridization conditions include overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in 0.1x SSC at about 65°C. Thus the present invention also includes polynucleotides obtainable by screening an appropriate library under stingent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or 3 or a fragment thereof.

The skilled artisan will appreciate that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for the polypeptide is short at the 5' end of the cDNA.

There are several methods available and well known to those skilled in the art to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman et al., PNAS USA 85, 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the 'missing' 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using 'nested' primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction can then be analysed by DNA sequencing and a full-length cDNA constructed either by joining the

product directly to the existing cDNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to an expression system which comprises a polynucleotide of the present invention, to host cells which are genetically engineered with such expression sytems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986) and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). Preferred such methods include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Preferably the proteins of the invention are coexpressed with thioredoxin in trans (TIT). Coexpression of thioredoxin in trans versus in cis is preferred to keep antigen free of thioredoxin without the need for protease. Thioredoxin coexpression eases the solubilisation of the proteins of the invention. Thioredoxin coexpression has also a significant impact on protein purification yield, on purified-protein solubility and quality.

Representative examples of appropriate hosts include bacterial cells, such as *Streptococci*, *Staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells;

animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (supra). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses.

Therefore, in certain embodiments, polynucleotides encoding immunogenic polypeptides of the present invention are introduced into suitable mammalian host cells for expression using any of a number of known viral-based systems. In one illustrative embodiment, retroviruses provide a convenient and effective platform for gene delivery systems. A selected nucleotide sequence encoding a polypeptide of the present invention can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to a subject. A number of

illustrative retroviral systems have been described (e.g., U.S. Pat. No. 5,219,740; Miller and Rosman (1989) BioTechniques 7:980-990; Miller, A. D. (1990) Human Gene Therapy 1:5-14; Scarpa et al. (1991) Virology 180:849-852; Burns et al. (1993) Proc. Natl. Acad. Sci. USA 90:8033-8037; and Boris-Lawrie and Temin (1993) Cur. Opin. Genet. Develop. 3:102-109.

In addition, a number of illustrative adenovirus-based systems have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham (1986) J. Virol. 57:267-274; Bett et al. (1993) J. Virol. 67:5911-5921; Mittereder et al. (1994) Human Gene Therapy 5:717-729; Seth et al. (1994) J. Virol. 68:933-940; Barr et al. (1994) Gene Therapy 1:51-58; Berkner, K. L. (1988) BioTechniques 6:616-629; and Rich et al. (1993) Human Gene Therapy 4:461-476).

Various adeno-associated virus (AAV) vector systems have also been developed for polynucleotide delivery. AAV vectors can be readily constructed using techniques well known in the art. See, e.g., U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 and WO 93/03769; Lebkowski et al. (1988) Molec. Cell. Biol. 8:3988-3996; Vincent et al. (1990) Vaccines 90 (Cold Spring Harbor Laboratory Press); Carter, B. J. (1992) Current Opinion in Biotechnology 3:533-539; Muzyczka, N. (1992) Current Topics in Microbiol. and Immunol. 158:97-129; Kotin, R. M. (1994) Human Gene Therapy 5:793-801; Shelling and Smith (1994) Gene Therapy 1:165-169; and Zhou et al. (1994) J. Exp. Med. 179:1867-1875.

Additional viral vectors useful for delivering the nucleic acid molecules encoding polypeptides of the present invention by gene transfer include those derived from the pox family of viruses, such as vaccinia virus and avian poxvirus. By way of example, vaccinia virus recombinants expressing the novel molecules can be constructed as follows. The DNA encoding a polypeptide is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells

which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the polypeptide of interest into the viral genome. The resulting TK.sup.(-) recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

A vaccinia-based infection/transfection system can be conveniently used to provide for inducible, transient expression or coexpression of one or more polypeptides described herein in host cells of an organism. In this particular system, cells are first infected in vitro with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide or polynucleotides of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into polypeptide by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation products. See, e.g., Elroy-Stein and Moss, Proc. Natl. Acad. Sci. USA (1990) 87:6743-6747; Fuerst et al. Proc. Natl. Acad. Sci. USA (1986) 83:8122-8126.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the coding sequences of interest. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an Avipox vector is particularly desirable in human and other mammalian species since members of the Avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant Avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, e.g., WO 91/12882; WO 89/03429; and WO 92/03545.

Any of a number of alphavirus vectors can also be used for delivery of polynucleotide compositions of the present invention, such as those vectors described in U.S. Patent Nos.

5,843,723; 6,015,686; 6,008,035 and 6,015,694. Certain vectors based on Venezuelan Equine Encephalitis (VEE) can also be used, illustrative examples of which can be found in U.S. Patent Nos. 5,505,947 and 5,643,576.

Moreover, molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al. J. Biol. Chem. (1993) 268:6866-6869 and Wagner et al. Proc. Natl. Acad. Sci. USA (1992) 89:6099-6103, can also be used for gene delivery under the invention.

Additional illustrative information on these and other known viral-based delivery systems can be found, for example, in Fisher-Hoch et al., *Proc. Natl. Acad. Sci. USA 86*:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci. 569*:86-103, 1989; Flexner et al., *Vaccine 8*:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques 6*:616-627, 1988; Rosenfeld et al., *Science 252*:431-434, 1991; Kolls et al., *Proc. Natl. Acad. Sci. USA 91*:215-219, 1994; Kass-Eisler et al., *Proc. Natl. Acad. Sci. USA 90*:11498-11502, 1993; Guzman et al., *Circulation 88*:2838-2848, 1993; and Guzman et al., *Cir. Res. 73*:1202-1207, 1993.

The recombinant live microorganisms described above can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

In certain embodiments, a polynucleotide may be integrated into the genome of a target cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the polynucleotide may be stably maintained in the cell as a separate, episomal segment of DNA. Such polynucleotide segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle.

The manner in which the expression construct is delivered to a cell and where in the cell the polynucleotide remains is dependent on the type of expression construct employed.

In another embodiment of the invention, a polynucleotide is administered/delivered as "naked" DNA, for example as described in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

In still another embodiment, a composition of the present invention can be delivered via a particle bombardment approach, many of which have been described. In one illustrative example, gas-driven particle acceleration can be achieved with devices such as those manufactured by Powderject Pharmaceuticals PLC (Oxford, UK) and Powderject Vaccines Inc. (Madison, WI), some examples of which are described in U.S. Patent Nos. 5,846,796; 6,010,478; 5,865,796; 5,584,807; and EP Patent No. 0500 799. This approach offers a needle-free delivery approach wherein a dry powder formulation of microscopic particles, such as polynucleotide or polypeptide particles, are accelerated to high speed within a helium gas jet generated by a hand held device, propelling the particles into a target tissue of interest.

In a related embodiment, other devices and methods that may be useful for gas-driven needle-less injection of compositions of the present invention include those provided by Bioject, Inc. (Portland, OR), some examples of which are described in U.S. Patent Nos. 4,790,824; 5,064,413; 5,312,335; 5,383,851; 5,399,163; 5,520,639 and 5,993,412.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for

refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

Another important aspect of the invention relates to a method for inducing , re-inforcing or modulating an immunological response in a mammal which comprises inoculating the mammal with a fragment or the entire polypeptide or polynucleotide of the invention, adequate to produce antibody and/or T cell immune response for prophylaxis or for therapeutic treatment of cancer and autoimmune disease and related conditions. Yet another aspect of the invention relates to a method of inducing, re-inforcing or modulating immunological response in a mammal which comprises, delivering a polypeptide of the present invention *via* a vector or cell directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce immune responses for prophylaxis or treatment of said mammal from diseases.

A further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces, re-inforces or modulates an immunological response in that mammal to a polypeptide of the present invention wherein the composition comprises a polypeptide or polynucleotide of the invention or an immunological fragment thereof as herein before defined.The vaccine formulation may further comprise a suitable carrier. Since a polypeptide may be broken down in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

A further aspect of the invention relates to the *in vitro* induction of immune responses to a fragment or the entire polypeptide or polynucleotide of the present invention or a

molecule comprising the polypeptide or polynucleotide of the present invention, using cells from the immune system of a mammal, and reinfusing these activated immune cells of the mammal for the treatment of disease. Activation of the cells from the immune system is achieved by in vitro incubation with the entire polypeptide or polynucleotide of the present invention or a molecule comprising the polypeptide or polynucleotide of the present invention in the presence or absence of various immunomodulator molecules. A further aspect of the invention relates to the immunization of a mammal by administration of antigen presenting cells modified by *in vitro* loading with part or the entire polypeptide of the present invention or a molecule comprising the polypeptide of the present invention and administered *in vivo* in an immunogenic way. Alternatively, antigen presenting cells can be transfected *in vitro* with a vector containing a fragment or the entire polynucleotide of the present invention or a molecule comprising the polynucleotide of the present invention, such as to express the corresponding polypeptide, and administered *in vivo* in an immunogenic way.

According to another embodiment, the pharmaceutical compositions described herein will comprise one or more immunostimulants in addition to the immunogenic polynucleotide, polypeptide, antibody, T-cell and/or antigen presenting cell (APC) compositions of this invention. An immunostimulant refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. One preferred type of immunostimulant comprises an adjuvant. Many adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Certain adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may also be used as adjuvants.

Within certain embodiments of the invention, the adjuvant composition is preferably one that induces an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (*e.g.*, IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (*e.g.*, IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Certain preferred adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL® adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science 273*:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins . Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A, β-escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamelar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol^R to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one preferred embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL® adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. Another

particularly preferred adjuvant formulation employing QS21, 3D-MPL® adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 as disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Additional illustrative adjuvants for use in the pharmaceutical compositions of the invention include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), **Detox (Enhanzyn®)** (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula (I):

$HO(CH_2CH_2O)_n$-A-R

Wherein, n is 1-50, A is a bond or –C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein *n* is between 1 and 50, preferably 4-24, most preferably 9; the *R* component is C_{1-50}, preferably C_4-C_{20} alkyl and most preferably C_{12} alkyl, and *A* is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-steoryl ether, polyoxyethylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl

ether are described in the Merck index (12th edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1μg - 200μg, such as 10-100μg, preferably 10μg - 50μg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

According to another embodiment of this invention, an immunogenic composition described herein is delivered to a host via antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature 392*:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman and Levy, *Ann. Rev. Med. 50*:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel et al., *Nature Med. 4*:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.*, CD54 and CD11) and costimulatory molecules (*e.g.*, CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide of the invention (or portion or other variant thereof) such that the encoded polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a pharmaceutical composition comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., *Immunology and cell Biology* 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the tumor polypeptide,

DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (*e.g.*, vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (*e.g.*, a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will typically vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, mucosal, intravenous, intracranial, intraperitoneal, subcutaneous and intramuscular administration.

Carriers for use within such pharmaceutical compositions are biocompatible, and may also be biodegradable. In certain embodiments, the formulation preferably provides a relatively constant level of active component release. In other embodiments, however, a more rapid rate of release immediately upon administration may be desired. The formulation of such compositions is well within the level of ordinary skill in the art using known techniques. Illustrative carriers useful in this regard include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other illustrative delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.*, a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

In another illustrative embodiment, biodegradable microspheres (*e.g.*, polylactate polyglycolate) are employed as carriers for the compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344, 5,407,609 and

5,942,252. Modified hepatitis B core protein carrier systems. such as described in WO/99 40934, and references cited therein, will also be useful for many applications. Another illustrative carrier/delivery system employs a carrier comprising particulate-protein complexes, such as those described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

The pharmaceutical compositions of the invention will often further comprise one or more buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate.

The pharmaceutical compositions described herein may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are typically sealed in such a way to preserve the sterility and stability of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation, is well known in the art, some of which are briefly discussed below for general purposes of illustration.

In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be

enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (see, for example, Mathiowitz *et al.*, Nature 1997 Mar 27;386(6623):410-4; Hwang *et al.*, Crit Rev Ther Drug Carrier Syst 1998;15(3):243-84; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451). Tablets, troches, pills, capsules and the like may also contain any of a variety of additional components, for example, a binder, such as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations will contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally. Such approaches are well known to the skilled artisan, some of which are further described, for example, in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363. In certain embodiments, solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally will contain a preservative to prevent the growth of microorganisms.

Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (for example, see U. S. Patent 5,466,468). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for

example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

In one embodiment, for parenteral administration in an aqueous solution, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. Moreover, for human administration, preparations will of course preferably meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

In another embodiment of the invention, the compositions disclosed herein may be formulated in a neutral or salt form. Illustrative pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation,

solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

The carriers can further comprise any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described, *e.g.,* in U. S. Patent 5,756,353 and U. S. Patent 5,804,212. Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, J Controlled Release 1998 Mar 2;52(1-2):81-7) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871) are also well-known in the pharmaceutical arts. Likewise, illustrative transmucosal drug delivery in the form of a polytetrafluoroetheylene support matrix is described in U. S. Patent 5,780,045.

In certain embodiments, liposomes, nanocapsules, microparticles, lipid particles, vesicles, and the like, are used for the introduction of the compositions of the present invention into suitable host cells/organisms. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like. Alternatively, compositions of the present invention can be bound, either covalently or non-covalently, to the surface of such carrier vehicles.

The formation and use of liposome and liposome-like preparations as potential drug carriers is generally known to those of skill in the art (see for example, Lasic, Trends Biotechnol 1998 Jul;16(7):307-21; Takakura, Nippon Rinsho 1998 Mar;56(3):691-5; Chandran *et al.*, Indian J Exp Biol. 1997 Aug;35(8):801-9; Margalit, Crit Rev Ther Drug Carrier Syst. 1995;12(2-3):233-61; U.S. Patent 5,567,434; U.S. Patent 5,552,157; U.S. Patent 5,565,213; U.S. Patent 5,738,868 and U.S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally difficult to transfect by other procedures, including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, J Biol Chem. 1990 Sep 25;265(27):16337-42; Muller *et al.*, DNA Cell Biol. 1990 Apr;9(3):221-9). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, various drugs, radiotherapeutic agents, enzymes, viruses, transcription factors, allosteric effectors and the like, into a variety of cultured cell lines and animals. Furthermore, he use of liposomes does not appear to be associated with autoimmune responses or unacceptable toxicity after systemic delivery.

In certain embodiments, liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs).

Alternatively, in other embodiments, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (see, for example, Quintanar-Guerrero *et al.*, Drug Dev Ind Pharm. 1998 Dec;24(12):1113-28). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) may be designed using polymers able to be degraded *in vivo*. Such particles can be made as described, for example, by Couvreur *et al.*, Crit Rev Ther Drug Carrier Syst. 1988;5(1):1-20; zur Muhlen *et al.*, Eur J Pharm Biopharm. 1998

Mar;45(2):149-55; Zambaux *et al.* J Controlled Release. 1998 Jan 2;50(1-3):31-40; and U. S. Patent 5,145,684.

This invention also relates to the use of polynucleotides, in the form of primers derived from the polynucleotides of the present invention, and of polypeptides, in the form of antibodies or reagents specific for the polypeptide of the present invention, as diagnostic reagents.

The identification of genetic or biochemical markers in blood or tissues that will enable the detection of very early changes along the carcinogenesis pathway will help in determining the best treatment for the patient. Surrogate tumour markers, such as polynucleotide expression, can be used to diagnose different forms and states of cancer. The identification of expression levels of the polynucleotides of the invention will be useful in both the staging of the cancerous disorder and grading the nature of the cancerous tissue. The staging process monitors the advancement of the cancer and is determined on the presence or absence of malignant tissue in the areas biopsied. The polynucleotides of the invention can help to perfect the staging process by identifying markers for the aggresivity of a cancer, for example the presence in different areas of the body. The grading of the cancer describes how closely a tumour resembles normal tissue of its same type and is assessed by its cell morphology and other markers of differentiation. The polynucleotides of the invention can be useful in determining the tumour grade as they can help in the determination of the differentiation status of the cells of a tumour.

The diagnostic assays offer a process for diagnosing or determining a susceptibility to cancers, autoimmune disease and related conditions through diagnosis by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of polypeptide or mRNA. This method of diagnosis is known as differential expression. The expression of a particular gene is compared between a diseased tissue and a normal tissue. A difference between the polynucleotide-related gene, mRNA, or protein in the two tissues is compared, for example in molecular weight, amino acid or nucleotide sequence, or relative abundance, indicates a change in the gene, or a gene which regulates it, in the tissue of the human that was suspected of being diseased.

Decreased or increased expression can be measured at the RNA level. PolyA RNA is first isolated from the two tissues and the detection of mRNA encoded by a gene corresponding to a differentially expressed polynucleotide of the invention can be detected by, for example, in situ hybridization in tissue sections, reverse trascriptase-PCR, using Northern blots containing poly A+ mRNA, or any other direct or inderect RNA detection method. An increased or decreased expression of a given RNA in a diseased tissue compared to a normal tissue suggests that the transcript and/or the expressed protein has a role in the disease. Thus detection of a higher or lower level of mRNA corresponding to SEQ ID NO 1 or 3 relative to normal level is indicative of the presence of cancer in the patient.

mRNA expression levels in a sample can be determined by generation of a library of expressed sequence tags (ESTs) from the sample. The relative representation of ESTs in the library can be used to assess the relative representation of the gene transcript in the starting sample. The EST analysis of the test can then be compared to the EST analysis of a reference sample to determine the relative expression levels of the polynucleotide of interest.

Other mRNA analyses can be carried out using serial analysis of gene expression (SAGE) methodology (Velculescu et. Al. Science (1995) 270:484) , differential display methodology (For example, US 5,776,683) or hybridization analysis which relies on the specificity of nucleotide interactions.

Alternatively, the comparison could be made at the protein level. The protein sizes in the two tissues may be compared using antibodies to detect polypeptides in Western blots of protein extracts from the two tissues. Expression levels and subcellular localization may also be detected immunologically using antibodies to the corresponding protein. Further assay techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. A raised or decreased level of polypeptide expression in the diseased tissue compared with the same protein expression level in the normal tissue indicates that the expressed protein may be involved in the disease.

In the assays of the present invention, the diagnosis can be determined by detection of gene product expression levels encoded by at least one sequence set forth in SEQ ID NOS: 1 or 3. A comparison of the mRNA or protein levels in a diseased versus normal tissue may also be used to follow the progression or remission of a disease.

A large number of polynucleotide sequences in a sample can be assayed using polynucleotide arrays. These can be used to examine differential expression of genes and to determine gene function. For example, arrays of the polynucleotide sequences SEQ ID NO: 1 or 3 can be used to determine if any of the polynucleotides are differentially expressed between a normal and cancer cell. In one embodiment of the invention, an array of oligonucleotides probes comprising the SEQ ID NO: 1 or 3 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

"Diagnosis" as used herein includes determination of a subject's susceptibility to a disease, determination as to whether a subject presently has the disease, and also the prognosis of a subject affected by the disease.

The present invention, further relates to a diagnostic kit for performing a diagnostic assay which comprises:

(a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO: 1 or 3, or a fragment thereof;

(b) a nucleotide sequence complementary to that of (a);

(c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO: 2 or 4, or a fragment thereof; or

(d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2 OR 4.

The nucleotide sequences of the present invention are also valuable for chromosomal localisation. The sequence is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined.

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them, can also be used as immunogens to produce antibodies immunospecific for polypeptides of the present invention. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

In a further aspect the invention provides an antibody immunospecific for a polypeptide according to the invention or an immunological fragment thereof as hereinbefore defined. Preferably the antibody is a monoclonal antibody

Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.
The antibody of the invention may also be employed to prevent or treat cancer, particularly ovarian and colon cancer, autoimmune disease and related conditions.

Another aspect of the invention relates to a method for inducing or modulating an immunological response in a mammal which comprises inoculating the mammal with a polypeptide of the present invention, adequate to produce antibody and/or T cell immune response to protect or ameliorate the symptoms or progression of the disease. Yet another aspect of the invention relates to a method of inducing or modulating immunological response in a mammal which comprises, delivering a polypeptide of the present invention *via* a vector directing expression of the polynucleotide and coding for the polypeptide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

It will be appreciated that the present invention therefore provides a method of treating abnormal conditions such as, for instance, cancer and autoimmune diseases, in particular, ovarian and colon cancer, related to either a presence of, an excess of, or an under-expression of, CASB6411 polypeptide activity.

The present invention further provides for a method of screening compounds to identify those which stimulate or which inhibit the function of the CASB6411 polypeptide. In general, agonists or antagonists may be employed for therapeutic and prophylactic purposes for such diseases as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such agonists, antagonists or inhibitors so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; or may be structural or functional mimetics thereof (see Coligan *et al.*, Current Protocols in

Immunology 1(2):Chapter 5 (1991)). Screening methods will be known to those skilled in the art. Further screening methods may be found in for example D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995) and references therein.

Thus the invention provides a method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of the invention which comprises a method selected from the group consisting of:

(a) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

(b) measuring the binding of a candidate compound to the polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presense of a labeled competitior;

(c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;

(d) mixing a candidate compound with a solution containing a polypeptide of claim 1, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or

(e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

The polypeptide of the invention may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. Well known screening methods may also be used to identify agonists and antagonists of the polypeptide of the invention which compete with the binding of the polypeptide of the invention to its receptors, if any.

Thus, in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for polypeptides of the present invention; or compounds which decrease or enhance the production of such polypeptides, which comprises:

(a) a polypeptide of the present invention;

(b) a recombinant cell expressing a polypeptide of the present invention;

(c) a cell membrane expressing a polypeptide of the present invention; or

(d) antibody to a polypeptide of the present invention;

which polypeptide is preferably that of SEQ ID NO:2 OR 4.

It will be readily appreciated by the skilled artisan that a polypeptide of the present invention may also be used in a method for the structure-based design of an agonist, antagonist or inhibitor of the polypeptide, by:

(a) determining in the first instance the three-dimensional structure of the polypeptide;

(b) deducing the three-dimensional structure for the likely reactive or binding site(s) of an agonist, antagonist or inhibitor;

(c) synthesing candidate compounds that are predicted to bind to or react with the deduced binding or reactive site; and

(d) testing whether the candidate compounds are indeed agonists, antagonists or inhibitors.

Gene therapy may also be employed to effect the endogenous production of CASB6411 polypeptide by the relevant cells in the subject. For an overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenum Press, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees.

Such amount will vary depending upon which specific immunogen is employed. Generally, it is expected that each dose will comprise 1-1000μg of protein, preferably 2-100μg, most preferably 4-40μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive a boost in about 4 weeks.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double stranded regions.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant

that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math., 48:* 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research *12(1):* 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., J. Molec. Biol. *215:* 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894; Altschul, S., *et al.*, J. Mol. Biol. *215*: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

The preferred algorithm used is FASTA. The preferred parameters for polypeptide or polynuleotide sequence comparison using this algorithm include the following:
Gap Penalty:12
Gap extension penalty: 4
Word size: 2, max 6

Preferred parameters for polypeptide sequence comparison with other methods include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polynucleotide comparisons.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the

numerical percent of the respective percent identity(divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%,etc., and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n. Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 OR 4 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2 OR 4, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 OR 4 by the numerical percent of the respective percent identity(divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:2 OR 4, or:

$$n_a \leq x_a - (x_a \bullet y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2 OR 4, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a subject sequence. Such relatedness may be quntified by determining the degree of identity and/or similarity between the sequences being compared as hereinbefore described. Falling within this generic term are the terms "ortholog", meaning a polynucleotide or polypeptide that is the functional equivalent of a polynucleotide or polypeptide in another species and "paralog" meaning a functionally similar sequence when considered within the same species.

Examples

Example 1

Subtractive cDNA cloning of colon tumour-associated antigen (TAA) candidates.

Subtractive cDNA libraries are produced using standard technologies. Briefly, total RNA is extracted from frozen (-70°C) tumour and matched normal colon samples using the TriPure reagent and protocol (Boehringer). Target RNA is prepared by pooling total RNA from three tumour samples (30 µg each). Driver RNA is prepared by pooling total RNA from three matched normal colon samples (10 µg each) and total RNA from seven normal tissues other than colon(brain, heart, kidney, liver, bladder, skin, spleen; 10 µg each). Total RNA from non-colon normal tissues is purchased from InVitrogen.

Messenger RNA is purified from total RNA using oligo-dT magnetic bead technology (Dynal) and quantified by spectrofluorimetry (BioRad).

Target and driver mRNA are reverse transcribed into cDNA using one of two strategies: 1) Target sequences for PCR oligonucleotides are introduced onto the ends of the newly synthesised cDNA during reverse transcription using the template switching capability of reverse transcriptase (ClonTech SMART PCR cDNA synthesis kit). 2) Alternatively, the target and driver mRNA are reverse transcribed into cDNA using an oligo-dT primer and converted to double-strand cDNA; the cDNA is cleaved with RsaI and linkers for PCR amplification are ligated onto the extremities of the cDNA fragments.

In both cases, target and driver cDNA are amplified by long range PCR (ClonTech SMART PCR Synthesis Kit and Advantage PCR Polymerase Mix) and used as starting material for subtractive cloning. For amplification, cycling conditions and optimisation of the number of PCR cycles are as described in the Advantage PCR protocol.

Two subtractive cloning strategies are used: ClonTech PCR SELECT (see ClonTech kit protocol and N. Gurskaya *et al.* 1996. Analytical Biochemistry: 240, 90) and cRDA(M. Hubank and D. Schatz. 1994. Nucleic Acids Research: 22, 5640) . When the PCR SELECT protocol is used, the primary PCR SELECT subtraction products are submitted to a supplementary round of cRDA subtraction. When the cRDA protocol is used, two consecutive cycles of cRDA subtraction are performed. In each case the products of both cycles of subtraction are cloned into pCR-TOPO (InVitrogen) and transformed into E. coli to produce a subtracted cDNA plasmid library.

An alternative strategy is also followed: subtraction of normal colon sequences and sequences from non-colon normal tissues are subtracted in separate hybridizations. In this case, target and driver RNA are assembled for the first subtraction as above with the exception that non-colon RNA is left out of the driver pool and amounts of normal colon are increased to 10 µg. Preparation of target and driver cDNA and subtractive hybridization are performed as described above. A second subtraction is then performed on the products of the first subtraction, but the driver is now composed of a pool of normal colon and normal non-colon mRNA from the seven normal tissues.

Example 2

Differential Screening of cDNA arrays.

Identification of tumour-associated genes in the subtracted cDNA library is accomplished by differential screening.

Total bacterial DNA is extracted from 100 µl over-night cultures. Bacteria are lysed with guanidium isothiocyantate and the bacterial DNA is affinity purified using magnetic glass (Boehringer). Plasmid inserts are recovered from the bacterial DNA by Advantage PCR amplification (Clontech). The PCR products are dotted onto two nylon membranes to produce high density cDNA arrays using the Biomek 96 HDRT tool (Beekman). The spotted cDNA is covalently linked to the membrane by UV irradiation. The first membrane is hybridised with a mixed cDNA probe prepared from the tumour of a single patient. The second membrane is hybridised with an equivalent amount of mixed cDNA probe prepared from normal colon of the same patient. The probe cDNA is prepared by PCR amplification as described above and is labelled using the AlkPhos Direct System (Amersham).

Hybridisation conditions and stringency washes are as described in the AlkPhos Direct kit. Hybridized probe is detected by chemiluminescence. Hybridisation intensities for each cDNA fragment on both blots are measured by film densitometry or direct measurement (BioRad Fluor-S Max). The ratio of the tumour to normal hybridisation intensities (T/N) is calculated for each gene to evaluate the degree of over-expression in the tumour. Genes which are significantly over-expressed in colon tumours are followed-up. Significance is arbitrarily defined as one standard deviation of the T/N frequency distribution. Differential screening experiments are repeated using RNA from multiple patient donors (>18) to estimate the frequency of over-expressing tumours in the patient population.

In addition, the DNA arrays are hybridised with mixed cDNA probes from normal tissues other than colon (see list above) to determine the level of expression of the candidate gene in these tissues.

Example 3

Real-time RT-PCR analysis

Real-time RT-PCR (U. Gibson. 1996. Genome Research: 6,996) is used to compare mRNA transcript abundance of the candidate antigen in matched tumour and normal colon tissues from multiple patients. In addition, mRNA levels of the candidate gene in a panel of normal tissues are also evaluated by this approach.

Total RNA from normal and tumour colon is extracted from snap frozen biopsies using TriPure reagent (Boehringer). Total RNA from normal tissues is purchased from InVitrogen or is extracted from snap frozen biopsies using TriPure reagent. Poly-A+ mRNA is purified from total RNA after DNAase treatment using oligo-dT magnetic beads (Dynal). Quantification of the mRNA is performed by spectrofluorimetry (VersaFluor, BioRad) using SybrII dye (Molecular Probes). Primers for real-time PCR amplification are designed with the Perkin-Elmer Primer Express software using default options for TaqMan amplification conditions.

Real-time reactions are assembled according to standard PCR protocols using 2 ng of purified mRNA for each reaction. SybrI dye (Molecular Probes) is added at a final dilution of 1/75000 for real-time detection. Amplification (40 cycles) and real-time detection is performed in a Perkin-Elmer Biosystems PE7700 system using conventional

instrument settings. Ct values are calculated using the PE7700 Sequence Detector software. Several Ct values are obtained for each samples : for the patient samples, the tumour Ct (CtT) and the matched normal colon Ct (CtN) values on the candidate TAA, and for the panel of normal tissue samples, a CtXY for each normal tissue XY. An another Ct (CtA) is also calculated on Actin gene, as an internal reference, for all of the samples.

As the efficiency of PCR amplification under the prevailing experimental conditions is close to the theoretical amplification efficiency, $2^{(CtN/T/XY-CtA)}$ value is an estimate of the relative TAA transcript level of the sample, standardised with respect to Actin transcript level. A value of 1 thus suggests the candidate antigen and Actin have the same expression level.

Real-time PCR reactions were performed on tumour colon and adjacent normal colon from biopsies of 18 patients. Results are shown in figure 1. 36 normal tissue samples, representing 28 different tissues (see table 2), were also tested by the same procedure. Results are shown in figure 2.

TAA transcript levels are calculated as described above. The proportion of patients over-expressing the candidate antigen, as well as the average transcript over-expression versus normal tissues is also calculated from this data set.
Overall results are shown in Table 1 :

Table 1 : CASB6411 Real-time PCR expression results

% of patients with a CASB6411 transcript level higher in tumour colon than adjacent normal colon (positive patients)	**83%**
% of positive patients with a CASB6411 transcript level at least 3 fold higher CASB6411 transcript than adjacent normal colon	**60%**
% of positive patients with a CASB6411 transcript level at least 10 fold higher in tumour colon than adjacent normal colon	**40%**
Average transcript over-expression fold in positive patients	**40**
% of patients with a CASB6411 transcript level higher in tumour colon than normal tissue median	**100%**
% of patients with a mRNA level at least 3 fold higher in tumour colon than normal tissue median	**100%**

% of patients with a mRNA level at least 10 fold higher in tumour colon than normal tissue median	40%
Normal tissues where CASB6411 transcript expression is equivalent than tumour transcript level in tumours	Lung, prostate

Table 1 clearly suggest CASB6411 transcript is over-expressed in colorectal tumours compared to adjacent normal colon and to most of the above mentioned normal tissues. More than 80% of the patients strongly over-express CASB6411 transcript in tumour, as compared to adjacent normal colon. Average over-expression fold is at least of 40, with 40 % of patients having overexpressing at least 10 fold. More over, all of the patients over-express CASB6411 transcript in colorectal tumors, as compared to other normal tissues, 40 % of them overexpressing it at least 10 fold.

Table 2 : listing of normal tissues used for CASB7439 transcript expression analysis.

Tissue	Abbreviation	Categorie
Adrenal gland	Ad_Gl	non dispensable
Aorta	Ao	non dispensable
Bladder	Bl	non dispensable
Bone marrow	Bo_Ma	non dispensable
Brain	Bra	non dispensable
Cervix	Ce	non dispensable
Colon	Co	non dispensable
Fallopian tube	Fa_Tu	non dispensable
Heart	He	non dispensable
Ileon	Il	non dispensable
Kidney	Ki	non dispensable
Liver	Li	non dispensable
Lung	Lu	non dispensable
Lymph node	Ly_No	non dispensable
Oesophagus	Oe	non dispensable
Parathyroid gland	Pa_Thy	non dispensable
Rectum	Re	non dispensable
Skin	Sk	non dispensable
Skeletal muscle	Sk_Mu	non dispensable
Small intestine	Sm_In	non dispensable
Spleen	Sp	non dispensable
Stomach	St	non dispensable
Thyroid gland	Thy	non dispensable
Trachea	Tra	non dispensable
Ovary	Ov	dispensable
Placenta	Pl	dispensable
Prostate	Pr	dispensable
T stis	Te	dispensable

Example 4

DNA microarrays

DNA micro-arrays are used to examine mRNA expression profiles of large collections of genes in multiple samples. This information is used to complement the data obtained by real-time PCR and provides an independent measure of gene expression levels in tumors and normal tissues.

Examples of current technologies for production of DNA micro-arrays include 1) The Affymetrix "GeneChip" arrays in which oligonucleotides are synthetized on the surface of the chip by solid phase chemical synthesis using a photolithographic process 2) DNA spotting technology in which small volumes of a DNA solution are robotically deposited and then immobilized onto the surface of a solid phase (e.g. glass). In both instances, the chips are hybridized with cDNA or cRNA which has been extracted from the tissue of interest (e.g. normal tissue, tumour etc...) and labeled with radioactivity or with a fluorescent reporter molecule. The labeled material is hybridized to the chip and the amount of probe bound to each sequence on the chip is determined using a specialized scanner. The experiment can be set-up with a single fluorescent reporter (or radioactivity) or, alternatively, can be performed using two fluorescent reporters. In this latter case, each of the two samples is labeled with one of the reporter molecules. The two labeled samples are then hybridized competitively to the sequences on the DNA chip. The ratio of the two fluorescent signals is determined for each sequence on the chip. This ratio is used to calculate the relative abundance of the transcript in the two samples. Detailed protocols are available from a number of sources including "DNA Microarrays: A practical approach. Schena M. Oxford University Press 1999" and the World Wide Web (http://cmgm.stanford.edu/pbrown/protocols/index.html), http://arrayit.com/DNA-Microarray-Protocols/) and specialized distributors (e.g. Affymetrix).

Example 5

Northern-Southern blot analysis

Limited amounts of mixed tumour and matched normal colon cDNA are amplified by Advantage PCR (see above). Messenger RNA from multiple normal tissues is also amplified using the same procedure. The amplified cDNA (1 μg) is electrophoresed on a

1.2% agarose gel and transferred onto a nylon membrane. The membrane is hybridised (AlkPhos Direct System) with a probe prepared using a fragment of the candidate TAA cDNA. Northern-Southern analysis provides information on transcript size, presence of splice variants and transcript abundance in tumour and normal tissues.

Example 6

Northern Blot Analysis

Northern blots are produced according to standard protocols using 1 µg of poly A+ mRNA. Radioactive probes are prepared using the Ready-to-Go system (Pharmacia).

Example 7

In silico detection of the full length cDNA sequence

EST sequence databases are screened with experimentally obtained cDNA sequence fragments, using the Blast algorithm (Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-41). The aim is to search for overlapping or longer identical EST sequences. Matched EST sequences are then assembled together, using the SeqMan software of the Lasergene package (DNASTAR). The consensus sequence of the resulting assembly is an EST-derived longer cDNA. This EST-derived cDNA is further analysed using the GeneMark software to find a potential open reading frame (ORF). The translated sequence of the ORF is compared with protein databases, using the Blast algorithm, to find homologues. If any, the homologous protein sequences are further used to complete the cDNA prediction by searching for genomic contig homologies using the Wise2 algorithm, leading to a genome-derived, virtual cDNA sequence. This virtual cDNA in finally assembled with EST-derived cDNA, and the new consensus cDNA undergoes a final check against ESTs to confirm the Wise2 prediction, and correct potential sequencing errors and frameshifts. The virtual cDNA is considered as a virtual full length cDNA once a full ORF (from start to stop codons), with clear protein homologies and coding potential.

The SEQ ID NO:1 has been obtained using this *in silico* cloning method of the full length cDNA sequence of CASB6411, which has a putative open reading frame of 460 amino acids (SEQ ID NO:2), and a potential isoform generated by alternative splicing (SEQ ID NO:3), encoding a truncated protein (SEQ ID NO:4).

Virtual full-length cDNA sequence is experimentally checked as described below.

Example 8

Experimental Identification of the full length cDNA sequence

Colon tumour cDNA libraries are constructed using the Lambda Zap II system (Stratagene) from 5 µg of polyA+ mRNA. The supplied protocol is followed except that SuperscriptII (Life Technologies) is used for the reverse transcription step. Oligo dT-primed and random-primed libraries are constructed. About 1.5 x106 independent phages are plated for each screening of the library. Phage plaques are transferred onto nylon filters and hybridised using a cDNA probe labelled with AlkPhos Direct. Positive phages are detected by chemiluminescence. Positive phage are excised from the agar plat, eluted in 500µl SM buffer and confirmed by gene-specific PCR. Eluted phages are converted to single strand M13 bacteriophage by in vivo excision. The bacteriophage is then converted to double strand plasmid DNA by infection of E. coli. Infected bacteria are plated and submitted to a second round of screening with the cDNA probe. Plasmid DNA is purified from positive bacterial clones and sequenced on both strands.

When the full length gene cannot be obtained directly from the cDNA library, missing sequence is isolated using RACE technology (Marathon Kit, ClonTech.). This approach relies on reverse transcribing mRNA into double strand cDNA, ligating linkers onto the ends of the cDNA and amplifying the desired extremity of the cDNA using a gene-specific primer and one of the linker oligonucleotides. Marathon PCR products are cloned into a plasmid (pCRII-TOPO, InVitrogen) and sequenced.

Example 9.

EST profiles

A complementary approach to experimental antigen tissue expression characterization is to explore the human EST database. ESTs ('Expressed Sequence Tags) are small fragments of cDNA made from a collection of mRNA extracted from a particular tissue or cell line. Such database currently provides a massive amount of human ESTs (10^6) from several hundreds of cDNA tissue libraries, including tumoral tissues from various types and states of disease. By means of informatics tools (Blast), a comparison search of

the CASB6411 sequence is performed in order to have further insight into tissue expression.

EST distribution of CASB6411 :

EST GenBank Accession number	EST cDNA tissue library
C00562	Human adult (K.Okubo)
C05837	Human pancreatic islet
AA172076	Stratagene ovarian cancer (#937219)
AA172244	Stratagene ovarian cancer (#937219)
AA612697	NCI_CGAP_Co10
AA371314	Prostate gland I
AI264367	NCI_CGAP_Co8
AI270207	NCI_CGAP_Co14
AI278830	NCI_CGAP_Co8
AI281230	NCI_CGAP_Co8
AI283827	NCI_CGAP_Co8
AI285194	NCI_CGAP_Co8
AI285227	NCI_CGAP_Co8
AI346622	NCI_CGAP_Co8
AI362363	NCI_CGAP_Gas4
AI473464	NCI_CGAP_Gas4
AI697014	NCI_CGAP_Pan1
AI799626	NCI_CGAP_Gas4
AI830044	NCI_CGAP_Lu19
AI921465	NCI_CGAP_Gas4
AI925050	NCI_CGAP_Gas4
AW029127	NCI_CGAP_Gas4
AW365013	DT0057
AW452356	NCI_CGAP_Sub5
AW469177	NCI_CGAP_Gas4
AW469181	NCI_CGAP_Gas4
AW582253	ST0212
AW810203	ST0125
AW810268	ST0125
AW810418	ST0125
AW814058	ST0198
AW869793	SN0075

SEQ ID NO:1 perfectly aligns with 32 ESTs : 13 are from 2 stomach cDNA libraries, 9 are from 3 tumor colon libraries, 2 are from one tumor ovary library, one is from one tumor pancreas library, one is from one lung tumor library, one is from one mixed tumor library, one is from one normal prostate library, one is from one normal stomach library, one is from normal pancreas library, 2 are from libraries of unknown type.

This clearly suggests, as expected, that CASB6411 is **over-expressed in tumor tissues**, with an emphasis in **colorectal and stomach tumor tissues**, as compared to normal tissues.

Example 10 :

10.1 Expression and purification of tumour-specific antigens

Expression in microbial hosts, or alternatively in vitro transcription/translation, is used to produce the antigen of the invention for vaccine purposes and to produce protein fragments or whole protein for rapid purification and generation of antibodies needed for characterization of the naturally expressed protein by immunohistochemistry or for follow-up of purification.

Recombinant proteins may be expressed in two microbial hosts, E. coli and in yeast (such as Saccharomyces cerevisiae or Pichia pastoris). This allows the selection of the expression system with the best features for this particular antigen production. In general, the recombinant antigen will be expressed in E. coli and the reagent protein expressed in yeast.

The expression strategy first involves the design of the primary structure of the recombinant antigen. In general an expression fusion partner (EFP) is placed at the N terminal extremity to improve levels of expression that could also include a region useful for modulating the immunogenic properties of the antigen, an immune fusion partner (IFP). In addition, an affinity fusion partner (AFP) useful for facilitating further purification is included at the C-terminal end.

When the recombinant strains are available, the recombinant product is characterized by the evaluation of the level of expression and the prediction of further solubility of the protein by analysis of the behavior in the crude extract.

After growth on appropriate culture medium and induction of the recombinant protein expression, total extracts are analyzed by SDS-PAGE. The recombinant proteins are visualized in stained gels and identified by Western blot analysis using specific antibodies.

A comparative evaluation of the different versions of the expressed antigen will allow the selection of the most promising candidate that is to be used for further purification and immunological evaluation.

The purification scheme follows a classical approach based on the presence of an His affinity tail in the recombinant protein. In a typical experiment the disrupted cells are filtered and the acellular extracts loaded onto an Ion Metal Affinity Chromatography (IMAC; Ni++NTA from Qiagen) that will specifically retain the recombinant protein. The retained proteins are eluted by 0-500 mM Imidazole gradient (possibly in presence of a detergent) in a phosphate buffer.

10.2 Antibody production and immunohistochemistry

Small amounts of relatively purified protein can be used to generate immunological tools in order to

a) detect the expression by immunohistochemistry in normal or cancer tissue sections;

b) detect the expression, and to follow the protein during the purification process (ELISA/ Western Blot); or

c) characterise/ quantify the purified protein (ELISA).

10.2.1 Polyclonal antibodies:

Immunization

Rabbits are immunised , intramuscularly (I.M.) , 3 times at 3 weeks intervals with 100µg of protein, formulated in the adjuvant 3D-MPL/QS21. Three weeks after each immunisation a blood sample is taken and the antibody titer estimated in the serum by ELISA using the protein as coating antigen following a standard protocol.

ELISA

96 well microplates (maxisorb Nunc) are coated with 5µg of protein overnight at 4°C. After 1hour saturation at 37°C with PBS NCS 1%, serial dilution of the rabbit sera is added for 1H 30 at 37°C (starting at 1/10). After 3 washings in PBS Tween, anti rabbit biotinylated anti serum (Amersham) is added (1/5000). Plates are washed and peroxydase coupled streptavidin (1/5000) is added for 30 min at 37°C. After washing, 50µl TMB (BioRad) is added for 7 min and the reaction then stopped with H2SO4 0.2M. The OD can be measured at 450 nm and midpoint dilutions calculated by SoftmaxPro.

10.2.2 Monoclonal antibodies:

Immunization

5 BALB/c mice are immunized 3 times at 3 week intervals with 5 μg of purified protein. Bleedings are performed 14 days post II and 1 week post 3. The sera are tested by Elisa on purified protein used as coated antigen. Based on these results (midpoint dilution > 10000) one mouse is selected for fusion

Fusion/ HATselection

Spleen cells are fused with the SP2/0 myeloma according to a standard protocol using PEG 40% and DMSO 5%. Cells are then seeded in 96 well plates 2.5 x104 - 105 cells/well and resistant clones will be selected in HAT medium. The supernatant of these hybridomas will be tested for their content in specific antibodies and when positive, will be submitted to 2 cycles of limited dilution . After 2 rounds of screening, 3 hybridomas will be chosen for ascitis production.

10.2.3 Immunohistochemistry

When antibodies are available, immuno staining is performed on normal or cancer tissue sections, in order to determine :

- ◊ the level of expression of the antigen of the invention in cancer relative to normal tissue or
- ◊ the proportion of cancer of a certain type expressing the antigen
- ◊ if other cancer types also express the antigen
- ◊ the proportion of cells expressing the antigen in a cancer tissue

Tissue sample preparation

After dissection, the tissue sample is mounted on a cork disk in OCT compound and rapidly frozen in isopentane previously super cooled in liquid nitrogen (-160°C). The block will then be conserved at -70°C until use. 7-10μm sections will be realised in a cryostat chamber (-20, -30°C).

Staining

Tissue sections are dried for 5 min at room Temperature (RT), fixed in acetone for 10min at RT, dried again, and saturated with PBS 0.5% BSA 5% serum. After 30 min at

RT either a direct or indirect staining is performed using antigen specific antibodies. A direct staining leads to a better specificity but a less intense staining whilst an indirect staining leads to a more intense but less specific staining.

10.3 Analysis of human cellular immune responses to the antigen of the invention

The immunological relevance of the antigen of the invention can be assessed by in vitro priming of human T cells. All T cell lymphocyte lines and dendritic cells are derived from PBMCs (peripheral blood mononuclear cells) of healthy donors (preferred HLA-A2 subtype). An HLA-A2.1/Kb transgenic mouse model is also used for screening of HLA-A2.1 peptides.

Newly discovered antigen-specific CD8+ T cell lines are raised and maintained by weekly in vitro stimulation. The lytic activity and the □-IFN production of the CD8 lines in response to the antigen or antigen derived-peptides is tested using standard procedures.

Two strategies to raise the CD8+ T cell lines are used: a peptide-based approach and a whole gene-based approach. Both approaches require the full-length cDNA of the newly discovered antigen in the correct reading frame to be either cloned in an appropriate delivery system or to be used to predict the sequence of HLA binding peptides.

Peptide-based approach

Briefly, transgenic mice are immunized with adjuvanted HLA-A2 peptides, those unable to induce a CD8 response (as defined by an efficient lysis of peptide-pulsed autologous spleen cells) will be further analyzed in the human system.

Human dendritic cells (cultured according to Romani et al.) will be pulsed with peptides and used to stimulate CD8-sorted T cells (by Facs). After several weekly stimulations, the CD8 lines will be first tested on peptide-pulsed autologous BLCL (EBV-B transformed cell lines). To verify the proper in vivo processing of the peptide, the CD8 lines will be tested on cDNA-transfected tumour cells (HLA-A2 transfected LnCaP, Skov3 or CAMA tumour cells).

Whole gene-based approach

CD8+ T cell lines will be primed and stimulated with either gene-gun transfected dendritic cells, retrovirally transduced B7.1-transfected fibroblasts, recombinant pox virus (Kim et al.) or adenovirus (Butterfield et al.) infected dendritic cells. Virus infected cells are very efficient to present antigenic peptides since the antigen is expressed at high level but can only be used once to avoid the over-growth of viral T cells lines.

After alternated stimulations, the CD8+ lines are tested on cDNA-transfected tumour cells as indicated above. Peptide specificity and identity is determined to confirm the immunological validation.

CD4+ T-cell response

Similarly, the CD4+ T-cell immune response can also be assessed. Generation of specific CD4+ T-cells is made using dendritic cells loaded with recombinant purified protein or peptides to stimulate the T-cells.

Predicted epitopes (nonamers and decamers) binding HLA alleles :

The HLA Class I binding peptide sequences are predicted either by the Parker's algorithm (Parker, K. C., M. A. Bednarek, and J. E. Coligan. 1994. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J. Immunol. 152:163 and http://bimas.dcrt.nih.gov/molbio/hla_bind/) or the Rammensee method (Rammensee, Friede, Stevanovic, MHC ligands and peptide motifs: 1st listing, Immunogenetics 41, 178-228, 1995 ; Rammensee, Bachmann, Stevanovic: MHC ligands and peptide motifs. Landes Bioscience 1997, and http://134.2.96.221/scripts/hlaserver.dll/home.htm). Peptides are then screened in the HLA-A2.1/Kb transgenic mice model (Vitiello et al.).

The HLA Class II binding peptide sequences are predicted using the Tepitope algorithm, with a score cut-off set to 6 (Sturniolo, Hammer at al., Nature Biotechnology. 1999. 17;555-561).

The following tables gather the Class I and II predicted epitope sequences :

HLA-A1 : decamers				
Rank	**Start Position**	**Subsequence Residue Listing**	**Parker's Score ***	**SEQ ID :**
1	379	ITEGRKIMIR	112.500	SEQ ID NO:9

HLA-A0201 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID :
1	191	LLMDFVFSL	27926.980	SEQ ID NO:10
2	292	FLLFFPSFT	2650.811	SEQ ID NO:11
3	285	QMMTFFIFL	2266.849	SEQ ID NO:12
4	286	MMTFFIFLL	1329.564	SEQ ID NO:13
5	403	FLIEKLIKL	926.658	SEQ ID NO:14
6	147	VLLIRNIFL	739.032	SEQ ID NO:15
7	240	LVWIGIFFC	450.023	SEQ ID NO:16
8	305	TLAITIWRL	368.501	SEQ ID NO:17
9	22	LIFCWDFTV	348.892	SEQ ID NO:18
10	203	FLGEFLRRI	343.941	SEQ ID NO:19
11	112	LLLPFVVSC	273.114	SEQ ID NO:20
12	66	LLTRFSAYM	176.513	SEQ ID NO:21
13	21	KLIFCWDFT	119.495	SEQ ID NO:22

5

HLA-A0201 : decamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID :
1	190	LLLMDFVFSL	6811.458	SEQ ID NO:23
2	191	LLMDFVFSLV	3407.985	SEQ ID NO:24
3	285	QMMTFFIFLL	2893.757	SEQ ID NO:25
4	93	YLAEYNLEFL	2497.344	SEQ ID NO:26
5	21	KLIFCWDFTV	1411.906	SEQ ID NO:27
6	293	LLFFPSFTGV	831.216	SEQ ID NO:28
7	65	QLLTRFSAYM	384.175	SEQ ID NO:29
8	239	TLVWIGIFFC	364.502	SEQ ID NO:30
9	284	SQMMTFFIFL	318.231	SEQ ID NO:31
10	111	VLLLPFVVSC	273.114	SEQ ID NO:32
11	162	ILCYYWLNTV	271.948	SEQ ID NO:33
12	366	TLIVLIITYL	270.234	SEQ ID NO:34
13	146	YVLLIRNIFL	174.977	SEQ ID NO:35
14	195	FVFSLVNSFL	174.977	SEQ ID NO:36
15	112	LLLPFVVSCI	150.931	SEQ ID NO:37
16	355	LIGSVHFFFI	147.808	SEQ ID NO:38
17	138	YEMPRHEVYV	126.598	SEQ ID NO:39
18	66	LLTRFSAYMV	118.238	SEQ ID NO:40

HLA A205 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID :
1	191	LLMDFVFSL	514.080	SEQ ID NO:10
2	403	FLIEKLIKL	126.000	SEQ ID NO:14

10

HLA A205 : decamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID
1	190	LLLMDFVFSL	171.360	SEQ ID NO:23
2	146	YVLLIRNIFL	126.000	SEQ ID NO:35
3	195	FVFSLVNSFL	126.000	SEQ ID NO:36
4	285	QMMTFFIFLL	100.800	SEQ ID NO:25

HLA A24 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID :
1	14	IYSGGITKL	220.000	SEQ ID NO:41
2	91	VYYLAEYNL	200.000	SEQ ID NO:42
3	165	YYWLNTVAL	200.000	SEQ ID NO:43
4	187	IYRLLLMDF	120.000	SEQ ID NO:44

HLA A24 : decamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score	SEQ ID :
1	145	VYVLLIRNIF	252.000	SEQ ID NO:45
2	164	CYYWLNTVAL	200.000	SEQ ID NO:46
3	92	YYLAEYNLEF	165.000	SEQ ID NO:47

HLA A3 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Parler's Score *	SEQ ID :
1	258	MLFIMFYSK	900.000	SEQ ID NO:48
2	148	LLIRNIFLK	135.000	SEQ ID NO:49
3	221	GLQEFDIAR	108.000	SEQ ID NO:50

- : Estimate of Half Time of Disassociation of a Molecule Containing This Subsequence.

HLA A3 : decamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID :
1	401	KMFLIEKLIK	600.000	SEQ ID NO:51
2	257	IMLFIMFYSK	270.000	SEQ ID NO:52
3	407	KLIKLQDMEK	180.000	SEQ ID NO:53
4	147	VLLIRNIFLK	135.000	SEQ ID NO:54

HLA B7 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID :
1	140	MPRHEVYVL	800.000	SEQ ID NO:55

HLA B7 : decamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID :

1	140	MPRHEVYVLL	800.000	SEQ ID NO:56

HLA B4403 nonamers				
Rank	Start Positi n	Subsequence Residue Listing	Parker's Score *	SEQ ID :
1	138	YEMPRHEVY	480.000	SEQ ID NO:57

HLA B4403 decamers				
Rank	Start Position	Subsequence Residue Listing	Parker's Score *	SEQ ID :
1	179	WETLIGQDIY	120.000	SEQ ID NO:58

*: Estimate of half time of disassociation of a molecule containing this subsequence.

HLA-DRB1*1501 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Tepitope Score	SEQ ID :
1	363	VHFFFILTL	6.5	SEQ ID NO:59
2	210	LRRIIGMQL	6.2	SEQ ID NO:60

HLA-DRB1*0301 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Tepitope Score	SEQ ID :
1	193	LLMDFVFSL	6.4	SEQ ID NO:61

HLA-DRB1*0703 : nonamers				
Rank	Start Position	Subsequence Residue Listing	Tepitope Score	SEQ ID :
1	216	MQLITSLGL	8.5	SEQ ID NO:62
2	197	FVFSLVNSF	8	SEQ ID NO:63
3	367	FILTLIVLI	8	SEQ ID NO:64
4	210	LRRIIGMQL	7.6	SEQ ID NO:60
5	381	WQITEGRKI	7.6	SEQ ID NO:65
6	148	VLLIRNIFL	7.3	SEQ ID NO:49
7	265	MFYSKNISL	7.3	SEQ ID NO:66
8	263	FIMFYSKNI	7.2	SEQ ID NO:67
9	305	VLCTLAITI	7.2	SEQ ID NO:17
10	284	WRASQMMTF	6.8	SEQ ID NO:68
11	365	FFFILTLIV	6.8	SEQ ID NO:69
12	133	FRLVERYEM	6.7	SEQ ID NO:70
13	366	FFILTLIVL	6.4	SEQ ID NO:71
14	238	YAQTLVWI	6.4	SEQ ID NO:72

SEQUENCE INFORMATION

SEQ ID NO:1

TGGGGAGGCAGAAGGCAGACTGATCACTTGAGGCCAGGAGTTTGAGACCTCAT
GTCTAAAAAAAAAAAAATTCTGTGAGGTGAGTTTTATTGTTATTCCCTCTCTACAG
ATATGGAAACTGAGGCTGAGAATCAGAACCATTCACAAGACAAAAATCCCCCAG
TTGGCAGATCCAGGGTTGCAAGCCAGGCCTGTGCAGCCCCAAAACCAGTGCTTG
TTTAACCACTGTGTGGTGACCACACCGCTCCAGGCCAACAGCTTGGGGCTAAGT
CTTCACGTTGCCTTTCACCATTAAATAATAGGGCTGCCCTTTGTTGAAGCCCTGC
ACTCCCAGTGACGGCCATAATAACCTTCAGGTGTTCTGCTTTCTGCCTTCTCTAG
Catggccaagtatttccggaacaacttcattaatccccacatttactccggagggatcaccaagctgatcttttgctgggacttcactg
tcactcatgaaaaagctgtgaagctaaaacagaagaatcttagcactgagataagggagaacctgtcagagctccgtcaggagaa
ttccaagttgacgttcaatcagctgctgacccgcttctctgcctacatggtagcctgggttgtctctacaggagtggccatagcctgctg
tgcagccgtttattacctggctgagtacaacttagagttcctgaagacacacagtaaccctggggcggtgctgttactgcctttcgttgt
gtcctgcattaatctggccgtgccatgcatctactccatgttcaggcttgtggagaggtacgagatgccacggcacgaagtctacgttc
tcctgatccgaaacatctttttgaaaatatcaatcattggcattctttgttactattggctcaacaccgtggccctgtctggtgaagagtg
ttgggaaaccctcattggccaggacatctaccggctccttctgatggattttgtgttctctttagtcaattccttcctgggggagtttctga
ggagaatcattgggatgcaactgatcacaagtcttggccttcaggagtttgacattgccaggaacgttctagaactgatctatgcaca
aactctggtgtggattggcatcttcttctgccccctgctgccctttatccaaatgattatgcttttcatcatgttctactccaaaaatatca
gcctgatgatgaatttccagcctccgagcaaagcctggcgggcctcacagatgatgacttttcttcatcttcttgctcttttttcccatcctt
caccggggtcttgtgcaccctggccatcaccatctggagattgaagccttcagctgactgtggccctttttcgaggtctgcctctcttcat
tcactccatctacagctggatcgacaccctaagtacacggcctggctacctgtgggttgtttggatctatcggaacctcattggaagtg
tgcacttctttttcatcctcaccctcattgtgctaatcatcacctatctttactggcagatcacagagggaaggaagattatgataaggc
tgctccatgagcagatcattaatgagggcaaagataaaatgttcctgatagaaaaattgatcaagctgcaggatatggagaagaaa
gcaaaccccagctcacttgttctggaaaggagagaggtggagcaacaaggctttttgcatttgggggaacatgatggcagtcttgac
ttgcgatctagaagatcagttcaagaaggtaatccaagggcctgaTGACTCTTTTGGTAACCAGACACCAA
TCAAATAAGGGGAGGAGACGAAAATGGAATGATTTCTTCCATGCCACCTGTGCC
TTTAGGAACTGCCCAGAAGAAAATCCAAGGCTTTAGCCAGGAGCGGAAACTGAC
TACCATGTAATTATCAAAGTAAAATTGGGCATTCCATGCTATTTTTAATACCTGG
ATTGCTGATTTTTCAAGACAAAATACTTGGGGTTTTCCAATAAAGATTGTTGTAA
TATTGAAATGAGCCTACAAAAACCTAGGAAGAGATAACTAGGGAATAATGTATA
TTATCTTCAAGAAATGTGTGCAGGAATGATTGGTTCTTAGAAATCTCTCCTGCCA
GACTTCCCAGACCTGGCAAAGGTTTAGAAACTGTTGCTAAGAAAAGTGGTCCAT
CCTGAATAAACATGTAATACTCCAGCAGGGATATGAAGCCTCTGAATTGTAGAA
CCTGCATTTATTTGTGACTTTGAACTAAAGACATCCCCCATGTCCCAAAGGTGG
AATACAACCAGAGGTCTCATCTCTGAACTTTCTTGCGTACTGATTACATGAGTCT

TTGGAGTCGGGGATGGAGGAGGTTCTGCCCCTGTGAGGTGTTATACATGACCAT
CAAAGTCCTACGTCAAGCT

SEQ ID NO:2

MAKYFRNNFINPHIYSGGITKLIFCWDFTVTHEKAVKLKQKNLSTEIRENLSELRQENSKLTFNQLLTRFSA
YMVAWVVSTGVAIACCAAVYYLAEYNLEFLKTHSNPGAVLLLPFVVSCINLAVPCIYSMFRLVERYEMPRHE
VYVLLIRNIFLKISIIGILCYYWLNTVALSGEECWETLIGQDIYRLLLMDFVFSLVNSFLGEFLRRIIGMQL
ITSLGLQEFDIARNVLELIYAQTLVWIGIFFCPLLPFIQMIMLFIMFYSKNISLMMNFQPPSKAWRASQMMT
FFIFLLFFPSFTGVLCTLAITIWRLKPSADCGPFRGLPLFIHSIYSWIDTLSTRPGYLWVVWIYRNLIGSVH
FFFILTLIVLIITYLYWQITEGRKIMIRLLHEQIINEGKDKMFLIEKLIKLQDMEKKANPSSLVLERREVEQ
QGFLHLGEHDGSLDLRSRRSVQEGNPRA

SEQ ID NO:3

TGGGGAGGCAGAAGGCAGACTGATCACTTGAGGCCAGGAGTTTGAGACCTCAT
GTCTAAAAAAAAAAAATTCTGTGAGGTGAGTTTTATTGTTATTCCCTCTCTACAG
ATATGGAAACTGAGGCTGAGAATCAGAACCATTCACAAGACAAAAATCCCCCAG
TTGGCAGATCCAGGGTTGCAAGCCAGGCCTGTGCAGCCCCAAAACCAGTGCTTG
TTTAACCACTGTGTGGTGACCACACCGCTCCAGGCCAACAGCTTGGGGCTAAGT
CTTCACGTTGCCTTTCACCATTAAATAATAGGGCTGCCCTTTGTTGAAGCCCTGC
ACTCCCAGTGACGGCCATAATAACCTTCAGGTGTTCTGCTTTCTGCCTTCTCTAG
Catggccaagtatttccggaacaacttcattaatccccacatttactccggagggatcaccaagctgatcttttgctgggacttcactg
tcactcatgaaaaagctgtgaagctaaaacagaagaatcttagcactgagataagggagaacctgtcagagctccgtcaggagaa
ttccaagttgacgttcaatcagctgctgacccgcttctctgcctacatggtagcctgggttgtctctacaggagtggccatagcctgctg
tgcagccgtttattacctggctgagtacaacttagagttcctgaagacacacagtaaccctggggcggtgctgttactgcctttcgttgt
gtcctgcattctggccgtgccatgcatctactccatgttcaggcttgtggagaggtacgagatgccacggcacgaagtctacgttctcc
tgatccgcaggggattgatgtagTTCTCAAGTATGGGATGTACAGATGGGCAGGCAGTGCAC
GCACAAAGGCTCCTGGGCTGAGGACGGGACTGAAATCATCCAGCGTTCCCCTTA
GTCAAGCTAAACATCTTTTTGAAAATATCAATCATTGGCATTCTTTGTTACTATT
GGCTCAACACCGTGGCCCTGTCTGGTGAAGAGTGTTGGGAAACCCTCATTGGCC
AGGACATCTACCGGCTCCTTCTGATGGATTTTGTGTTCTCTTTAGTCAATTCCTT
CCTGGGGGAGTTTCTGAGGAGAATCATTGGGATGCAACTGATCACAAGTCTTGG
CCTTCAGGAGTTTGACATTGCCAGGAACGTTCTAGAACTGATCTATGCACAAAC
TCTGGTGTGGATTGGCATCTTCTTCTGCCCCCTGCTGCCCTTTATCCAAATGATT
ATGCTTTTCATCATGTTCTACTCCAAAAATATCAGCCTGATGATGAATTTCCAGC

CTCCGAGCAAAGCCTGGCGGGCCTCACAGATGATGACTTTCTTCATCTTCTTGC
TCTTTTTCCCATCCTTCACCGGGGTCTTGTGCACCCTGGCCATCACCATCTGGA
GATTGAAGCCTTCAGCTGACTGTGGCCCTTTTCGAGGTCTGCCTCTCTTCATTC
ACTCCATCTACAGCTGGATCGACACCCTAAGTACACGGCCTGGCTACCTGTGGG
TTGTTTGGATCTATCGGAACCTCATTGGAAGTGTGCACTTCTTTTTCATCCTCAC
CCTCATTGTGCTAATCATCACCTATCTTTACTGGCAGATCACAGAGGGAAGGAA
GATTATGATAAGGCTGCTCCATGAGCAGATCATTAATGAGGGCAAAGATAAAAT
GTTCCTGATAGAAAAATTGATCAAGCTGCAGGATATGGAGAAGAAAGCAAACCC
CAGCTCACTTGTTCTGGAAAGGAGAGAGGTGGAGCAACAAGGCTTTTTGCATTT
GGGGGAACATGATGGCAGTCTTGACTTGCGATCTAGAAGATCAGTTCAAGAAG
GTAATCCAAGGGCCTGATGACTCTTTTGGTAACCAGACACCAATCAAATAAGGG
GAGGAGACGAAAATGGAATGATTTCTTCCATGCCACCTGTGCCTTTAGGAACTG
CCCAGAAGAAAATCCAAGGCTTTAGCCAGGAGCGGAAACTGACTACCATGTAAT
TATCAAAGTAAAATTGGGCATTCCATGCTATTTTTAATACCTGGATTGCTGATTT
TTCAAGACAAAATACTTGGGGTTTTCCAATAAAGATTGTTGTAATATTGAAATGA
GCCTACAAAAACCTAGGAAGAGATAACTAGGGAATAATGTATATTATCTTCAAG
AAATGTGTGCAGGAATGATTGGTTCTTAGAAATCTCTCCTGCCAGACTTCCCAG
ACCTGGCAAAGGTTTAGAAACTGTTGCTAAGAAAAGTGGTCCATCCTGAATAAA
CATGTAATACTCCAGCAGGGATATGAAGCCTCTGAATTGTAGAACCTGCATTTA
TTTGTGACTTTGAACTAAAGACATCCCCCATGTCCCAAAGGTGGAATACAACCA
GAGGTCTCATCTCTGAACTTTCTTGCGTACTGATTACATGAGTCTTTGGAGTCG
GGGATGGAGGAGGTTCTGCCCCTGTGAGGTGTTATACATGACCATCAAAGTCCT
ACGTCAAGCT

SEQ ID NO:4

MAKYFRNNFINPHIYSGGITKLIFCWDFTVTHEKAVKLKQKNLSTEIRENLSELRQE
NSKLTFNQLLTRFSAYMVAWVVSTGVAIACCAAVYYLAEYNLEFLKTHSNPGAVLL
LPFVVSCILAVPCIYSMFRLVERYEMPRHEVYVLLIRRGLM

SEQ ID NO:5

atcttttgctgggacttcactgtcactcatgaaaaagctgtgaagctaaaacagaagaatcttagcactgag
ataagggagaacctgtcagagctccgtcaggagaattccaagttgacgttcaatcagctgctgacccgcttc
tctgcctacatggtagcctgggttgtctctacaggagtggccatagcctgctgtgcagccgtttattacctg
gctgagtacaacttagagttcctgaagacacacagtaaccctggggcggtgctgttactgcctttcgttgtg
tcctgcattaatctggccgtgccatgcatctactccatgttcaggcttgtggagaggtacgagatgccacgg

cacgaagtctacgttctcctgatccgaaacatcttttgaaaatatcaatcattggcattctttgttactat
tggctcaacaccgtggccctgtctggtgaagagtgttgggaaaccctcattggccaggacatctaccggctc
cttctgatggattttgtgttctctttagtcaattccttcctgggggagtttctgaggagaatcattgggatg
caactgatcacaagtcttggccttcaggagtttgacattgccaggaacgttctagaactgatctatgcacaa
actctggtgtggattggcatcttcttctgccccctgctgcctttatccaaatgattatgcttttcatcatg
ttctactccaaaaatatcagcctgatgatgaatttccagcctccgagcaaagcctggcgggcctcacagatg
atgactttcttcatcttcttgctctttttcccatctttcaccggggtcttgtgcaccctggccatcaccatc
tggagattgaagccttcagctgactgtggccctttcgaggtctgcctctcttcattcactccatctacagc
tggatcgacaccctaagtacacggcctggctacctgtgggttgtttggatctatcggaacctcattggaagt
gtgcacttctttttcatcctcaccctcattgtgctgatcatcacctatctttactggcagatcacagaggga
aggaagattatgataaggctgctccatgagcagatcattaatgagggcaaagataaaatgttcctgatagaa
aaattgatcaagctgcaggatatggagaagaaagcaaaccccagctcacttgttctggaaaggagagaggtg
gagcaacaaggctttttgcatttgggggaacatgatggcagtcttgacttgcgatctagaagatcagttcaa
gaaggtaatccaagggcctgaTGACTCTTTTGGTAACCAGACACCAATCAAATAAGGGGAGGAGATGAAAAT
GGAATGATTTCTTCCATGCCACCTGTGCCTTTAGGAACTGCCCAGAAGAAAATCCAAGGCTTTAGCCAGGAG
CGGAAACTGACTACCATGTAATTATCAAAGTAAAATTGGGCATTCCATGCTATTTTTAATACCTGGATTGCT
GATTTTTCAAGACAAAATACTTGGGGTTTTCCAATAAAGATTGTTGTAATATTGAAATGAGCCTACAAAAAC
CTAGGAAGAGATAACTAGGGAATAATGTATATTATCTTCAAGAAATGTGTGCAGGAATGATTGGTTCTTAGA
AATCTCTCCTGCCAGACTTCCCAGACCTGGCAAAGGTTTAGAAACTGTTGCTAAGAAAAGTGGTCCATCCTG
AATAAACATGTAATACTCCAGCAGGGATATGAAGCCTCTGAATTGTAGAACCTGCATTTATTTGTGACTTTG
AACTAAAGACATCCCCCATGTCCCAAAGGTGGAATACAACCAGAGGTCTCATCTCTGAACTTTCTTGCGTAC
TGATTACATGAGTCTTTGGAGTCGGGGATGGAGGAGGTTCTGCCCCTGTGAGGTGTTATACATGACCATCAA
AGTCCTACGTCAAGCT

SEQ ID NO:6

IFCWDFTVTHEKAVKLKQKNLSTEIRENLSELRQENSKLTFNQLLTRFSAYMVAWVVSTGVAIACCAAVYYL
AEYNLEFLKTHSNPGAVLLLPFVVSCINLAVPCIYSMFRLVERYEMPRHEVYVLLIRNIFLKISIIGILCYY
WLNTVALSGEECWETLIGQDIYRLLLMDFVFSLVNSFLGEFLRRIIGMQLITSLGLQEFDIARNVLELIYAQ
TLVWIGIFFCPLLPFIQMIMLFIMFYSKNISLMMNFQPPSKAWRASQMMTFFIFLLFFPSFTGVLCTLĀITI
WRLKPSADCGPFRGLPLFIHSIYSWIDTLSTRPGYLWVVWIYRNLIGSVHFFFILTLIVLIITYLYWQITEG
RKIMIRLLHEQIINEGKDKMFLIEKLIKLQDMEKKANPSSLVLERREVEQQGFLHLGEHDGSLDLRSRRSVQ
EGNPRA

SEQ ID NO:7

ctgatgatgaatttccagcctccgagcaaagcctggcgggcctcacagatgatgactttcttcatcttcttgctctttttcccatctttcac
cggggtcttgtgcaccctggccatcaccatctggagattgaagccttcagctgactgtggccctttcgaggtctgcctctcttcattca
ctccatctacagctggatcgacaccctaagtacacggcctggctacctgtgggttgtttggatctatcggaacctcattggaagtgtgc

acttctttttcatcctcaccctcattgtgctgatcatcacctatctttactggcagatcacagagggaaggaagattatgataaggctgc
tccatgagcagatcattaatgagggcaaagataaaatgttcctgatagaaaaattgatcaagctgcaggatatggagaagaaagc
aaaccccagctcacttgttctggaaaggagagaggtggagcaacaaggctttttgcatttgggggaacatgatggcagtcttgactt
gcgatctagaagatcagttcaagaaggtaatccaagggcctgaTGACTCTTTTGGTAACCAGACACCAAT
CAAATAAGGGGAGGAGATGAAAATGGAATGATTTCTTCCATGCCACCTGTGCCT
TTAGGAACTGCCCAGAAGAAAATCCAAGGCTTTAGCCAGGAGCGGAAACTGACT
ACCATGTAATTATCAAAGTAAAATTGGGCATTCCATGCTATTTTTAATACCTGGA
TTGCTGATTTTTCAAGACAAAATACTTGGGGTTTTCCAATAAAGATTGTTGTAAT
ATTGAAATGAGCCTACAAAAACCTAGGAAGAGATAACTAGGGAATAATGTATAT
TATCTTCAAGAAATGTGTGCAGGAATGATTGGTTCTTAGAAATCTCTCCTGCCA
GACTTCCCAGACCTGGCAAAGGTTTAGAAACTGTTGCTAAGAAAAGTGGTCCAT
CCTGAATAAACATGTAATACTCCAGCAGGGATATGAAGCCTCTGAATTGTAGAA
CCTGCATTTATTTGTGACTTTGAACTAAAGACATCCCCCATGTCCCAAAGGTGG
AATACAACCAGAGGTCTCATCTCTGAACTTTCTTGCGTACTGATTACATGAGTCT
TTGGAGTCGGGGATGGAGGAGGTTCTGCCCCTGTGAGGTGTTATACATGACCAT
CAAAGTCCTACGTCAAGCT

SEQ ID NO:8

LMMNFQPPSKAWRASQMMTFFIFLLFFPSFTGVLCTLAITIWRLKPSADCGPFRGLPLFIHSIYSWIDTLST
RPGYLWVVWIYRNLIGSVHFFFILTLIVLIITYLYWQITEGRKIMIRLLHEQIINEGKDKMFLIEKLIKLQD
MEKKANPSSLVLERREVEQQGFLHLGEHDGSLDLRSRRSVQEGNPRA

SEQ ID NO:9

ITEGRKIMIR

SEQ ID NO:10

LLMDFVFSL

SEQ ID NO:11

FLLFFPSFT

SEQ ID NO:12

QMMTFFIFL

SEQ ID NO:13

MMTFFIFLL

SEQ ID NO:14

FLIEKLIKL

SEQ ID NO:15

VLLIRNIFL

SEQ ID NO:16

LVWIGIFFC

SEQ ID NO:17

TLAITIWRL

SEQ ID NO:18

LIFCWDFTV

SEQ ID NO:19

FLGEFLRRI

SEQ ID NO:20

LLLPFVVSC

SEQ ID NO:21

LLTRFSAYM

SEQ ID NO:22

KLIFCWDFT

SEQ ID NO:23

LLLMDFVFSL

SEQ ID NO:24

LLMDFVFSLV

SEQ ID NO:25

QMMTFFIFLL

SEQ ID NO:26

YLAEYNLEFL

SEQ ID NO:27

KLIFCWDFTV

SEQ ID NO:28

LLFFPSFTGV

SEQ ID NO:29

QLLTRFSAYM

SEQ ID NO:30

TLVWIGIFFC

SEQ ID NO:31

SQMMTFFIFL

SEQ ID NO:32

VLLLPFVVSC

SEQ ID NO:33

ILCYYWLNTV

SEQ ID NO:34

TLIVLIITYL

SEQ ID NO:35

YVLLIRNIFL

SEQ ID NO:36

FVFSLVNSFL

SEQ ID NO:37

LLLPFVVSCI

SEQ ID NO:38

LIGSVHFFFI

SEQ ID NO:39

YEMPRHEVYV

SEQ ID NO:40

LLTRFSAYMV

SEQ ID NO:41

IYSGGITKL

SEQ ID NO:42

VYYLAEYNL

SEQ ID NO:43

YYWLNTVAL

SEQ ID NO:44

IYRLLLMDF

SEQ ID NO:45

VYVLLIRNIF

SEQ ID NO:46

CYYWLNTVAL

SEQ ID NO:47

YYLAEYNLEF

SEQ ID NO:48

MLFIMFYSK

SEQ ID NO:49

LLIRNIFLK

SEQ ID NO:50

GLQEFDIAR

SEQ ID NO:51

KMFLIEKLIK

SEQ ID NO:52

IMLFIMFYSK

SEQ ID NO:53

KLIKLQDMEK

SEQ ID NO:54

VLLIRNIFLK

SEQ ID NO:55

MPRHEVYVL

SEQ ID NO:56

MPRHEVYVLL

SEQ ID NO:57

YEMPRHEVY

SEQ ID NO:58

WETLIGQDIY

SEQ ID NO:59

VHFFFILTL

SEQ ID NO:60

LRRIIGMQL

SEQ ID NO:61

LLMDFVFSL

SEQ ID NO:62

MQLITSLGL

SEQ ID NO:63

FVFSLVNSF

SEQ ID NO:64

FILTLIVLI

SEQ ID NO:65

WQITEGRKI

SEQ ID NO:66

MFYSKNISL

SEQ ID NO:67

FIMFYSKNI

SEQ ID NO:68

WRASQMMTF

SEQ ID NO:69

FFFILTLIV

SEQ ID NO:70

FRLVERYEM

SEQ ID NO:71

FFILTLIVL

SEQ ID NO:72

YAQTLVWI

Claims

1. An isolated polypeptide comprising an amino acid sequence which has at least 70% identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 over the entire length of of SEQ ID NO:2 or SEQ ID NO:4 respectively.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to SEQ ID NO:2 or SEQ ID NO:4.

3. The polypeptide as claimed in claim 1 comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

4. The isolated polypeptide of SEQ ID NO:2 or SEQ ID NO:4.

5. A polypeptide comprising an immunogenic fragment of a polypeptide as claimed in any one of claims 1 to 4 in which the immunogenic activity of the immunogenic fragment is substantially the same as the polypeptide of SEQ ID NO:2 or SEQ ID NO:4.

6. A polypeptide as claimed in any of claims 1 to 5 wherein said polypeptide is part of a larger fusion protein.

7. A polypeptide as claimed in any of claims 1 to 6 chemically conjugated to a carrier protein.

8. An isolated polynucleotide encoding a polypeptide as claimed in any of claims 1 to 6.

9. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 70% identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, over the entire length of SEQ ID NO:2 or SEQ ID NO:4 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

10. An isolated polynucleotide comprising a nucleotide sequence that has at least 70% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2 or SEQ ID NO:4,

over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

11. An isolated polynucleotide which comprises a nucleotide sequence which has at least 70% identity to that of SEQ ID NO:1 or SEQ ID NO:3 over the entire length of SEQ ID NO:1 or SEQ ID NO:3 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

12. The isolated polynucleotide as defined in any one of claims 8 to 11 in which the identity is at least 95%.

13. An isolated polynucleotide selected from:

(a) a polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or SEQ ID NO:4;

(b) the coding region of the polynucleotide of SEQ ID NO:1 or SEQ ID NO:3; and

(c) a polynucleotide obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1 or SEQ ID NO:3 or a fragment thereof said polynucleotide encoding a protein which has similar immunogenic properties to those of the protein of sequence ID NO:2 or SEQ ID NO:4 respectively or a nucleotide sequence complementary to said isolated polynucleotide

14. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 8 - 13.

15. A host cell comprising the expression vector of claim 14 or the isolated polynucleotide of claims 8 to 13.

16. A process for producing a polypeptide of claims 1 to 7 comprising culturing a host cell of claim 15 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

17. A vaccine comprising an effective amount of the polypeptide of any one of claims 1 to 7 and a pharmaceutically acceptable carrier.

18. A vaccine comprising an effective amount of the polynucleotide of any one of claims 8 to 13 and a pharmaceutically effective carrier.

19. A vaccine comprising an effective amount of antigen presenting cells, modified by in vitro loading with a polypeptide of any one of claims 1 to 7, or genetically modified in vitro to express a polypeptide of claims 1 to 7 and a pharmaceutically effective carrier.

20. A vaccine as claimed in any one of claims 17 to 19 which additionally comprises a TH-1 inducing adjuvant.

21. A vaccine as claimed in claim 20 in which the TH-1 inducing adjuvant is selected from the group of adjuvants comprising: 3D-MPL, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.

22. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

23. A method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of any one of claims 1 to 5 which comprises a method selected from the group consisting of:

(a) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

(b) measuring the binding of a candidate compound to the said polypeptide (or to the cells or membranes bearing the polypeptide) or a fusion protein thereof in the presence of a labeled competitior;

(c) testing whether the candidate compound results in a signal generated by activation or inhibition of the said polypeptide, using detection systems appropriate to the cells or cell membranes bearing the polypeptide;

(d) mixing a candidate compound with a solution containing a polypeptide of any one of claims 1 to 7, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a standard; or

(e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

24. A method for the treatment of a subject by immunoprophylaxis or therapy comprising *in vitro* induction of immune responses to a molecule of any one of claims 1 to 5, using *in vitro* incubation of the polypeptide of any one of claims 1 to 7 or the polynucleotide of any one of claims 8 to 13 with cells from the immune system of a mammal, and reinfusing these activated immune cells to the mammal for the treatment of disease.

25. A method as claimed in claim 24 wherein the treatment is for ovarian or colon cancer.

26. An agonist or antagonist to the polypeptide of claims 1 to 5.

27. A compound which is:
(a) an agonist or antagonist to the polypeptide of claims 1 to 5;
(b) isolated polynucleotide of claims 8 to 13; or
(c) a nucleic acid molecule that modulates the expression of the nucleotide sequence encoding the polypeptide of any one of claims 1 to 5;
for use in therapy.

28. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polypeptide of any one of claims 1 to 5 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

29. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of a polynucleotide of any one of claims 8 to 13 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

30. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polypeptide of any one of claims 1 to 5 in a subject comprising analyzing for the presence or amount of said polypeptide in a sample derived from said subject.

31. A process for diagnosing the presence of colon cancer or a susceptibility to colon cancer in a subject related to expression or activity of a polynucleotide of any one of claims 8 to 13 in a subject comprising analyzing for the presence or amount of said polynucleotide in a sample derived from said subject.

32. An isolated polynucleotide selected from the group consisting of:
(a) an isolated polynucleotide comprising a nucleotide sequence which has at least 70% identity to SEQ ID NO:5 or SEQ ID NO:7 over the entire length of SEQ ID NO:5 or SEQ ID NO:7 respectively;
(b) an isolated polynucleotide comprising the polynucleotide of SEQ ID NO:5 or SEQ ID NO:7;
(c) the polynucleotide of SEQ ID NO:5 or SEQ ID NO:7.

33. A live vaccine composition comprising an expression vector or recombinant live micro-organism according to claim 14.

34. Use of a polynucleotide as claimed in any one of claims 8 to 13 for the manufacture of a medicament in the treatment of carcinoma.

35. Use of a polynucleotide as claimed in any one of claims 8 to 13 for the manufacture of a medicament in the treatment of colon carcinoma.

36. Use of a polypeptide as claimed in any one of claims 1 to 7 for the manufacture of a medicament in the treatment of carcinoma.

37. Use of a polypeptide as claimed in any one of claims 1 to 7 for the manufacture of a medicament in the treatment of colon carcinoma.

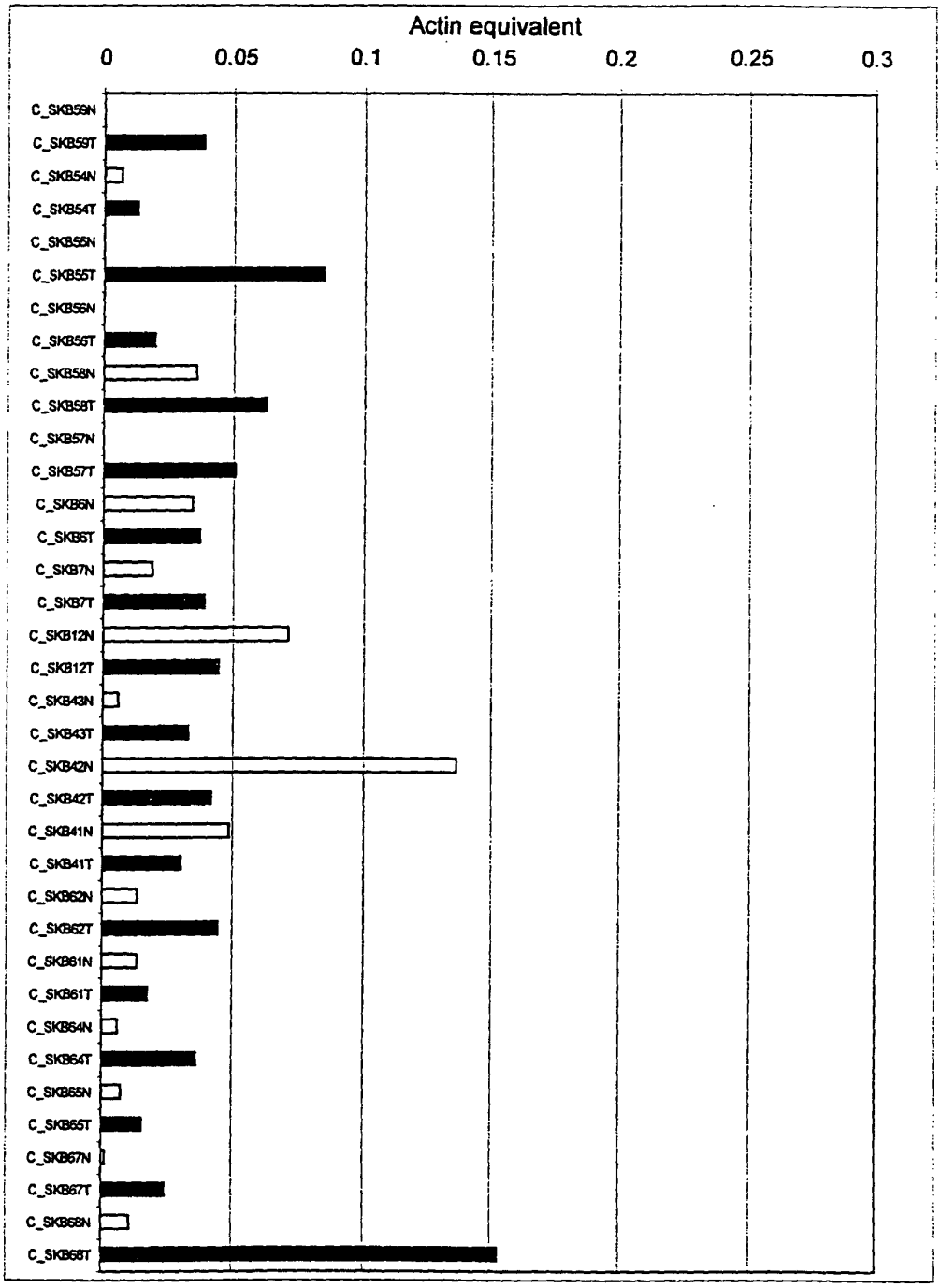
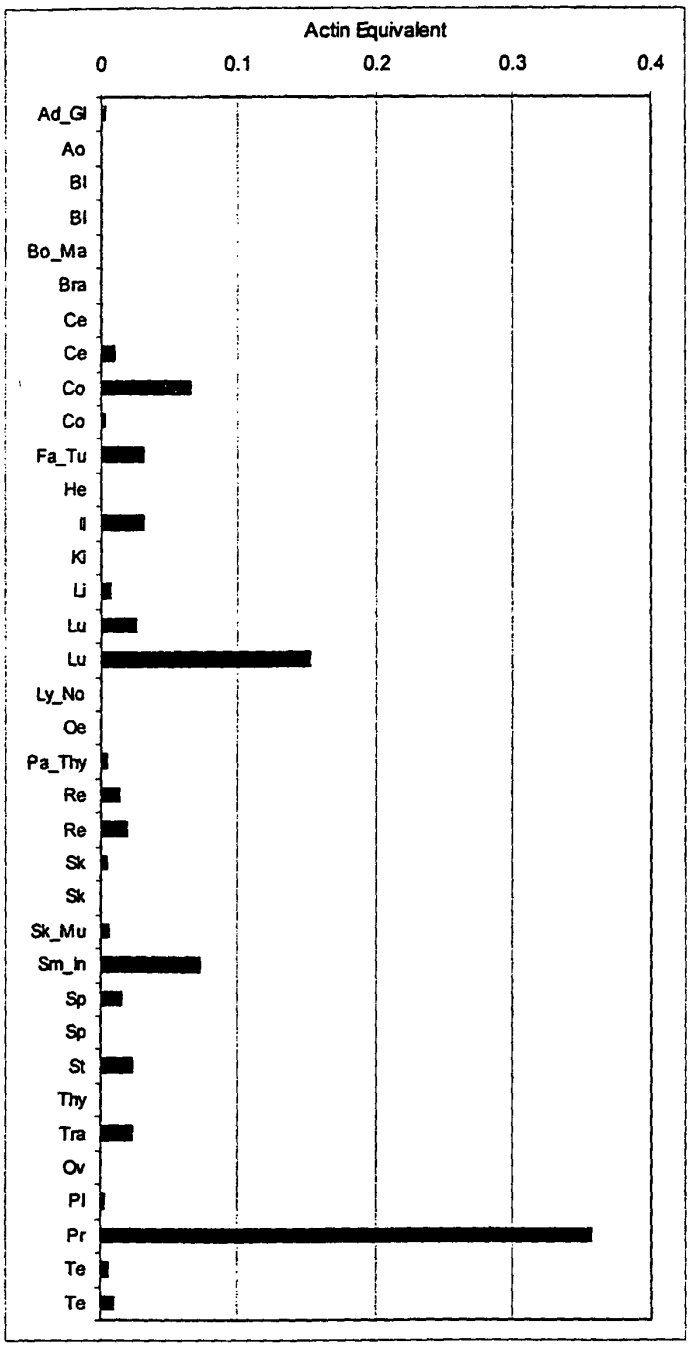


Figure 1: RT PCR data of CASB6411 transcript on coloractal tumours and matched normal colon.

5



Legend:
Ad_Gl: adrenal gland;
Ao: aorta; Bl: bladder,
Bo_Ma: bone marrow;
Bra: brain;
Ce: cervix; Co: colon;
Fa_Tu: fallopian tube; He: heart;
Il: ileon; Ki: kidney; Li: liver;
Lu: lung; Ly_No: lymph node;
Oe: oesophagus; Ov: ovary;
Pa_Thy: parathyroid gland;
Pl: placenta; Pr: prostate;
Re: rectum; Sk: skin;
Sk_Mu: skeletal muscle;
Sm_In: small intestine;
Sp: spleen; St: stomach; Te: testis;
Thy: thyroid gland; Tra: trachea;
Bre: Breast.

Figure 2: RT PCR data of CASB76411 transcript in a panel of normal tissues.

SEQUENCE LISTING

<110> SmithKline Beecham Biologicals

<120> Novel compounds

<130> BC45263

<160> 72

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 2407
<212> DNA
<213> Artificial Sequence

<400> 1

tggggaggca gaaggcagac tgatcacttg aggccaggag tttgagacct catgtctaaa 60
aaaaaaaaat tctgtgaggt gagttttatt gttattccct ctctacagat atggaaactg 120
aggctgagaa tcagaaccat tcacaagaca aaaatccccc agttggcaga tccagggttg 180
caagccaggc ctgtgcagcc ccaaaaccag tgcttgttta accactgtgt ggtgaccaca 240
ccgctccagg ccaacagctt ggggctaagt cttcacgttg cctttcacca ttaaataata 300
gggctgccct ttgttgaagc cctgcactcc cagtgacggc cataataacc ttcaggtgtt 360
ctgctttctg ccttctctag catggccaag tatttccgga acaacttcat taatccccac 420
atttactccg gagggatcac caagctgatc ttttgctggg acttcactgt cactcatgaa 480
aaagctgtga agctaaaaca gaagaatctt agcactgaga taagggagaa cctgtcagag 540
ctccgtcagg agaattccaa gttgacgttc aatcagctgc tgacccgctt ctctgcctac 600
atggtagcct gggttgtctc tacaggagtg gccatagcct gctgtgcagc cgtttattac 660
ctggctgagt acaacttaga gttcctgaag acacacagta accctggggc ggtgctgtta 720
ctgcctttcg ttgtgtcctg cattaatctg gccgtgccat gcatctactc catgttcagg 780
cttgtggaga ggtacgagat gccacggcac gaagtctacg ttctcctgat ccgaaacatc 840
tttttgaaaa tatcaatcat tggcattctt tgttactatt ggctcaacac cgtggccctg 900
tctggtgaag agtgttggga aaccctcatt ggccaggaca tctaccggct ccttctgatg 960
gattttgtgt tctctttagt caattccttc ctgggggagt ttctgaggag aatcattggg 1020
atgcaactga tcacaagtct tggccttcag gagtttgaca ttgccaggaa cgttctagaa 1080
ctgatctatg cacaaactct ggtgtggatt ggcatcttct tctgcccct gctgcccttt 1140
atccaaatga ttatgctttt catcatgttc tactccaaaa atatcagcct gatgatgaat 1200
ttccagcctc cgagcaaagc ctggcgggcc tcacagatga tgacttttctt catcttcttg 1260
ctctttttcc catccttcac cggggtcttg tgcaccctgg ccatcaccat ctggagattg 1320
aagccttcag ctgactgtgg ccctttttcga ggtctgcctc tcttcattca ctccatctac 1380
agctggatcg acaccctaag tacacggcct ggctacctgt gggttgtttg gatctatcgg 1440
aacctcattg gaagtgtgca cttctttttc atcctcaccc tcattgtgct aatcatcacc 1500
tatctttact ggcagatcac agagggaagg aagattatga taaggctgct ccatgagcag 1560
atcattaatg agggcaaaga taaaatgttc ctgatagaaa aattgatcaa gctgcaggat 1620
atggagaaga aagcaaaccc cagctcactt gttctggaaa ggagagaggt ggagcaacaa 1680
ggctttttgc atttgggga acatgatggc agtcttgact tgcgatctag aagatcagtt 1740
caagaaggta atccaagggc ctgatgactc ttttggtaac cagacaccaa tcaaataagg 1800
ggaggagacg aaaatggaat gatttcttcc atgccacctg tgcctttagg aactgcccag 1860
aagaaaatcc aaggctttag ccaggagcgg aaactgacta ccatgtaatt atcaaagtaa 1920
aattgggcat tccatgctat ttttaatacc tggattgctg atttttcaag acaaaatact 1980
tggggttttc caataaagat tgttgtaata ttgaaatgag cctacaaaaa cctaggaaga 2040
gataactagg gaataatgta tattatcttc aagaaatgtg tgcaggaatg attggttctt 2100
agaaatctct cctgccagac ttcccagacc tggcaaaggt ttagaaactg ttgctaagaa 2160
aagtggtcca tcctgaataa acatgtaata ctccagcagg gatatgaagc ctctgaattg 2220
tagaacctgc atttatttgt gactttgaac taaagacatc ccccatgtcc caaaggtgga 2280
atacaaccag aggtctcatc tctgaacttt cttgcgtact gattacatga gtctttggag 2340
tcggggatgg aggaggttct gcccctgtga ggtgttatac atgaccatca aagtcctacg 2400
tcaagct 2407

<210> 2
<211> 460
<212> PRT
<213> Artificial Sequence

<400> 2

```
Met Ala Lys Tyr Ph  Arg Asn Asn Phe Ile Asn Pro His Ile Tyr Ser
 1               5                  10                  15
Gly Gly Ile Thr Lys Leu Ile Phe Cys Trp Asp Phe Thr Val Thr His
            20                  25                  30
Glu Lys Ala Val Lys Leu Lys Gln Lys Asn Leu Ser Thr Glu Ile Arg
        35                  40                  45
Glu Asn Leu Ser Glu Leu Arg Gln Glu Asn Ser Lys Leu Thr Phe Asn
    50                  55                  60
Gln Leu Leu Thr Arg Phe Ser Ala Tyr Met Val Ala Trp Val Val Ser
65                  70                  75                  80
Thr Gly Val Ala Ile Ala Cys Cys Ala Ala Val Tyr Tyr Leu Ala Glu
                85                  90                  95
Tyr Asn Leu Glu Phe Leu Lys Thr His Ser Asn Pro Gly Ala Val Leu
            100                 105                 110
Leu Leu Pro Phe Val Val Ser Cys Ile Asn Leu Ala Val Pro Cys Ile
        115                 120                 125
Tyr Ser Met Phe Arg Leu Val Glu Arg Tyr Glu Met Pro Arg His Glu
    130                 135                 140
Val Tyr Val Leu Leu Ile Arg Asn Ile Phe Leu Lys Ile Ser Ile Ile
145                 150                 155                 160
Gly Ile Leu Cys Tyr Tyr Trp Leu Asn Thr Val Ala Leu Ser Gly Glu
                165                 170                 175
Glu Cys Trp Glu Thr Leu Ile Gly Gln Asp Ile Tyr Arg Leu Leu Leu
            180                 185                 190
Met Asp Phe Val Phe Ser Leu Val Asn Ser Phe Leu Gly Glu Phe Leu
        195                 200                 205
Arg Arg Ile Ile Gly Met Gln Leu Ile Thr Ser Leu Gly Leu Gln Glu
    210                 215                 220
Phe Asp Ile Ala Arg Asn Val Leu Glu Leu Ile Tyr Ala Gln Thr Leu
225                 230                 235                 240
Val Trp Ile Gly Ile Phe Phe Cys Pro Leu Leu Pro Phe Ile Gln Met
                245                 250                 255
Ile Met Leu Phe Ile Met Phe Tyr Ser Lys Asn Ile Ser Leu Met Met
            260                 265                 270
Asn Phe Gln Pro Pro Ser Lys Ala Trp Arg Ala Ser Gln Met Met Thr
        275                 280                 285
Phe Phe Ile Phe Leu Leu Phe Phe Pro Ser Phe Thr Gly Val Leu Cys
    290                 295                 300
Thr Leu Ala Ile Thr Ile Trp Arg Leu Lys Pro Ser Ala Asp Cys Gly
305                 310                 315                 320
Pro Phe Arg Gly Leu Pro Leu Phe Ile His Ser Ile Tyr Ser Trp Ile
                325                 330                 335
Asp Thr Leu Ser Thr Arg Pro Gly Tyr Leu Trp Val Val Trp Ile Tyr
            340                 345                 350
Arg Asn Leu Ile Gly Ser Val His Phe Phe Phe Ile Leu Thr Leu Ile
        355                 360                 365
Val Leu Ile Ile Thr Tyr Leu Tyr Trp Gln Ile Thr Glu Gly Arg Lys
    370                 375                 380
Ile Met Ile Arg Leu Leu His Glu Gln Ile Ile Asn Glu Gly Lys Asp
385                 390                 395                 400
Lys Met Phe Leu Ile Glu Lys Leu Ile Lys Leu Gln Asp Met Glu Lys
                405                 410                 415
Lys Ala Asn Pro Ser Ser Leu Val Leu Glu Arg Arg Glu Val Glu Gln
            420                 425                 430
Gln Gly Phe Leu His Leu Gly Glu His Asp Gly Ser Leu Asp Leu Arg
        435                 440                 445
Ser Arg Arg Ser Val Gln Glu Gly Asn Pro Arg Ala
    450                 455                 460
```

<210> 3
<211> 2521
<212> DNA
<213> Artificial Sequenc

<400> 3

tggggaggca gaaggcagac tgatcacttg aggccaggag tttgagacct catgtctaaa 60
aaaaaaaaat tctgtgaggt gagttttatt gttattccct ctctacagat atggaaactg 120
aggctgagaa tcagaaccat tcacaagaca aaaatccccc agttggcaga tccagggttg 180
caagccaggc ctgtgcagcc ccaaaaccag tgcttgttta accactgtgt ggtgaccaca 240
ccgctccagg ccaacagctt ggggctaagt cttcacgttg cctttcacca ttaaataata 300
gggctgccct ttgttgaagc cctgcactcc cagtgacggc cataataacc ttcaggtgtt 360
ctgctttctg ccttctctag catggccaag tatttccgga acaacttcat taatccccac 420
atttactccg gagggatcac caagctgatc ttttgctggg acttcactgt cactcatgaa 480
aaagctgtga agctaaaaca gaagaatctt agcactgaga taagggagaa cctgtcagag 540
ctccgtcagg agaattccaa gttgacgttc aatcagctgc tgacccgctt ctctgcctac 600
atggtagcct gggttgtctc tacaggagtg gccatagcct gctgtgcagc cgtttattac 660
ctggctgagt acaacttaga gttcctgaag acacacagta accctggggc ggtgctgtta 720
ctgcctttcg ttgtgtcctg cattctggcc gtgccatgca tctactccat gttcaggctt 780
gtggagaggt acgagatgcc acggcacgaa gtctacgttc tcctgatccg caggggattg 840
atgtagttct caagtatggg atgtacagat gggcaggcag tgcacgcaca aaggctcctg 900
ggctgaggac gggactgaaa tcatccagcg ttccccttag tcaagctaaa catcttttg 960
aaaatatcaa tcattggcat tctttgttac tattggctca acaccgtggc cctgtctggt 1020
gaagagtgtt gggaaaccct cattggccag gacatctacc ggctccttct gatggatttt 1080
gtgttctctt tagtcaattc cttcctgggg gagtttctga ggagaatcat tgggatgcaa 1140
ctgatcacaa gtcttggcct tcaggagttt gacattgcca ggaacgttct agaactgatc 1200
tatgcacaaa ctctggtgtg gattggcatc ttcttctgcc ccctgctgcc ctttatccaa 1260
atgattatgc ttttcatcat gttctactcc aaaaatatca gcctgatgat gaatttccag 1320
cctccgagca aagcctggcg ggcctcacag atgatgactt tcttcatctt cttgctcttt 1380
ttcccatcct tcaccggggt cttgtgcacc ctggccatca ccatctggag attgaagcct 1440
tcagctgact gtggcccttt tcgaggtctg cctctcttca ttcactccat ctacagctgg 1500
atcgacaccc taagtacacg gcctggctac ctgtgggttg tttggatcta tcggaacctc 1560
attggaagtg tgcacttctt tttcatcctc accctcattg tgctaatcat cacctatctt 1620
tactggcaga tcacagaggg aaggaagatt atgataaggc tgctccatga gcagatcatt 1680
aatgagggca aagataaaat gttcctgata gaaaaattga tcaagctgca ggatatggag 1740
aagaaagcaa accccagctc acttgttctg gaaaggagag aggtggagca acaaggcttt 1800
ttgcatttgg gggaacatga tggcagtctt gacttgcgat ctagaagatc agttcaagaa 1860
ggtaatccaa gggcctgatg actcttttgg taaccagaca ccaatcaaat aaggggagga 1920
gacgaaaatg gaatgatttc ttccatgcca cctgtgcctt taggaactgc ccagaagaaa 1980
atccaaggct ttagccagga gcggaaactg actaccatgt aattatcaaa gtaaaattgg 2040
gcattccatg ctatttttaa tacctggatt gctgattttt caagacaaaa tacttggggt 2100
tttccaataa agattgttgt aatattgaaa tgagcctaca aaaacctagg aagagataac 2160
tagggaataa tgtatattat cttcaagaaa tgtgtgcagg aatgattggt tcttagaaat 2220
ctctcctgcc agacttccca gacctggcaa aggtttagaa actgttgcta agaaaagtgg 2280
tccatcctga ataaacatgt aatactccag cagggatatg aagcctctga attgtagaac 2340
ctgcatttat ttgtgacttt gaactaaaga catcccccat gtcccaaagg tggaatacaa 2400
ccagaggtct catctctgaa ctttcttgcg tactgattac atgagtcttt ggagtcgggg 2460
atggaggagg ttctgcccct gtgaggtgtt atacatgacc atcaaagtcc tacgtcaagc 2520
t 2521

<210> 4
<211> 154
<212> PRT
<213> Artificial Sequence

<400> 4

Met Ala Lys Tyr Phe Arg Asn Asn Phe Ile Asn Pro His Ile Tyr Ser
1 5 10 15
Gly Gly Ile Thr Lys Leu Ile Phe Cys Trp Asp Phe Thr Val Thr His
20 25 30
Glu Lys Ala Val Lys Leu Lys Gln Lys Asn Leu Ser Thr Glu Ile Arg
35 40 45
Glu Asn Leu Ser Glu Leu Arg Gln Glu Asn Ser Lys Leu Thr Phe Asn
50 55 60
Gln Leu Leu Thr Arg Phe Ser Ala Tyr Met Val Ala Trp Val Val Ser
65 70 75 80
Thr Gly Val Ala Ile Ala Cys Cys Ala Ala Val Tyr Tyr Leu Ala Glu
85 90 95
Tyr Asn Leu Glu Phe Leu Lys Thr His Ser Asn Pro Gly Ala Val Leu
100 105 110
Leu Leu Pro Phe Val Val Ser Cys Ile Leu Ala Val Pro Cys Ile Tyr

```
        115                 120                 125
Ser Met Phe Arg Leu Val Glu Arg Tyr Glu Met Pro Arg His Glu Val
    130                 135                 140
Tyr Val Leu Leu Ile Arg Arg Gly Leu Met
145                 150
```

<210> 5
<211> 1960
<212> DNA
<213> Artificial Sequence

<400> 5

```
atcttttgct gggacttcac tgtcactcat gaaaaagctg tgaagctaaa acagaagaat      60
cttagcactg agataaggga gaacctgtca gagctccgtc aggagaattc caagttgacg     120
ttcaatcagc tgctgacccg cttctctgcc tacatggtag cctgggttgt ctctacagga     180
gtggccatag cctgctgtgc agccgtttat tacctggctg agtacaactt agagttcctg     240
aagacacaca gtaaccctgg ggcggtgctg ttactgcctt tcgttgtgtc ctgcattaat     300
ctggccgtgc catgcatcta ctccatgttc aggcttgtgg agaggtacga gatgccacgg     360
cacgaagtct acgttctcct gatccgaaac atctttttga aaatatcaat cattggcatt     420
ctttgttact attggctcaa caccgtggcc ctgtctggtg aagagtgttg ggaaaccctc     480
attggccagg acatctaccg gctccttctg atggattttg tgttctcttt agtcaattcc     540
ttcctggggg agtttctgag gagaatcatt gggatgcaac tgatcacaag tcttggcctt     600
caggagtttg acattgccag gaacgttcta gaactgatct atgcacaaac tctggtgtgg     660
attggcatct tcttctgccc cctgctgccc tttatccaaa tgattatgct tttcatcatg     720
ttctactcca aaaatatcag cctgatgatg aatttccagc ctccgagcaa agcctggcgg     780
gcctcacaga tgatgacttt cttcatcttc ttgctctttt tcccatcttt caccggggtc     840
ttgtgcaccc tggccatcac catctggaga ttgaagcctt cagctgactg tggccctttt     900
cgaggtctgc ctctcttcat tcactccatc tacagctgga tcgacaccct aagtacacgg     960
cctggctacc tgtgggttgt ttggatctat cggaacctca ttggaagtgt gcacttcttt    1020
ttcatcctca ccctcattgt gctgatcatc acctatcttt actggcagat cacagaggga    1080
aggaagatta tgataaggct gctccatgag cagatcatta atgagggcaa agataaaatg    1140
ttcctgatag aaaaattgat caagctgcag gatatggaga agaaagcaaa ccccagctca    1200
cttgttctgg aaaggagaga ggtggagcaa caaggctttt tgcatttggg ggaacatgat    1260
ggcagtcttg acttgcgatc tagaagatca gttcaagaag gtaatccaag ggcctgatga    1320
ctcttttggt aaccagacac caatcaaata aggggaggag atgaaaatgg aatgatttct    1380
tccatgccac ctgtgccttt aggaactgcc cagaagaaaa tccaaggctt tagccaggag    1440
cggaaactga ctaccatgta attatcaaag taaaattggg cattccatgc tatttttaat    1500
acctggattg ctgatttttc aagacaaaat acttggggtt ttccaataaa gattgttgta    1560
atattgaaat gagcctacaa aaacctagga agagataact agggaataat gtatattatc    1620
ttcaagaaat gtgtgcagga atgattggtt cttagaaatc tctcctgcca gacttcccag    1680
acctggcaaa ggtttagaaa ctgttgctaa gaaaagtggt ccatcctgaa taaacatgta    1740
atactccagc agggatatga agcctctgaa ttgtagaacc tgcatttatt tgtgactttg    1800
aactaaagac atcccccatg tcccaaaggt ggaatacaac cagaggtctc atctctgaac    1860
tttcttgcgt actgattaca tgagtctttg gagtcgggga tggaggaggt tctgcccctg    1920
tgaggtgtta tacatgacca tcaaagtcct acgtcaagct                          1960
```

<210> 6
<211> 438
<212> PRT
<213> artifical sequence

<400> 6

```
Ile Phe Cys Trp Asp Phe Thr Val Thr His Glu Lys Ala Val Lys Leu
 1               5                  10                  15
Lys Gln Lys Asn Leu Ser Thr Glu Ile Arg Glu Asn Leu Ser Glu Leu
            20                  25                  30
Arg Gln Glu Asn Ser Lys Leu Thr Phe Asn Gln Leu Leu Thr Arg Phe
        35                  40                  45
Ser Ala Tyr Met Val Ala Trp Val Val Ser Thr Gly Val Ala Ile Ala
    50                  55                  60
Cys Cys Ala Ala Val Tyr Tyr Leu Ala Glu Tyr Asn Leu Glu Phe Leu
65                  70                  75                  80
Lys Thr His Ser Asn Pro Gly Ala Val Leu Leu Leu Pro Phe Val Val
                85                  90                  95
Ser Cys Ile Asn Leu Ala Val Pro Cys Ile Tyr Ser Met Phe Arg Leu
            100                 105                 110
```

```
Val Glu Arg Tyr Glu Met Pro Arg His Glu Val Tyr Val Leu Leu Ile
        115                 120                 125
Arg Asn Ile Phe Leu Lys Ile Ser Ile Ile Gly Ile Leu Cys Tyr Tyr
    130                 135                 140
Trp Leu Asn Thr Val Ala Leu Ser Gly Glu Glu Cys Trp Glu Thr Leu
145                 150                 155                 160
Ile Gly Gln Asp Ile Tyr Arg Leu Leu Leu Met Asp Ph  Val Phe Ser
                165                 170                 175
Leu Val Asn Ser Phe Leu Gly Glu Phe Leu Arg Arg Ile Ile Gly Met
            180                 185                 190
Gln Leu Ile Thr Ser Leu Gly Leu Gln Glu Phe Asp Ile Ala Arg Asn
        195                 200                 205
Val Leu Glu Leu Ile Tyr Ala Gln Thr Leu Val Trp Ile Gly Ile Phe
    210                 215                 220
Phe Cys Pro Leu Leu Pro Phe Ile Gln Met Ile Met Leu Phe Ile Met
225                 230                 235                 240
Phe Tyr Ser Lys Asn Ile Ser Leu Met Met Asn Phe Gln Pro Pro Ser
                245                 250                 255
Lys Ala Trp Arg Ala Ser Gln Met Met Thr Phe Phe Ile Phe Leu Leu
            260                 265                 270
Phe Phe Pro Ser Phe Thr Gly Val Leu Cys Thr Leu Ala Ile Thr Ile
        275                 280                 285
Trp Arg Leu Lys Pro Ser Ala Asp Cys Gly Pro Phe Arg Gly Leu Pro
    290                 295                 300
Leu Phe Ile His Ser Ile Tyr Ser Trp Ile Asp Thr Leu Ser Thr Arg
305                 310                 315                 320
Pro Gly Tyr Leu Trp Val Val Trp Ile Tyr Arg Asn Leu Ile Gly Ser
                325                 330                 335
Val His Phe Phe Phe Ile Leu Thr Leu Ile Val Leu Ile Ile Thr Tyr
            340                 345                 350
Leu Tyr Trp Gln Ile Thr Glu Gly Arg Lys Ile Met Ile Arg Leu Leu
        355                 360                 365
His Glu Gln Ile Ile Asn Glu Gly Lys Asp Lys Met Phe Leu Ile Glu
    370                 375                 380
Lys Leu Ile Lys Leu Gln Asp Met Glu Lys Lys Ala Asn Pro Ser Ser
385                 390                 395                 400
Leu Val Leu Glu Arg Arg Glu Val Glu Gln Gln Gly Phe Leu His Leu
                405                 410                 415
Gly Glu His Asp Gly Ser Leu Asp Leu Arg Ser Arg Arg Ser Val Gln
            420                 425                 430
Glu Gly Asn Pro Arg Ala
        435
```

<210> 7
<211> 1219
<212> DNA
<213> Artificial Sequence

<400> 7

```
ctgatgatga atttccagcc tccgagcaaa gcctggcggg cctcacagat gatgactttc      60
ttcatcttct tgctcttttt cccatctttc accggggtct tgtgcaccct ggccatcacc     120
atctggagat tgaagccttc agctgactgt ggccctttcc gaggtctgcc tctcttcatt     180
cactccatct acagctggat cgacacccta agtacacggc ctggctacct gtgggttgtt     240
tggatctatc ggaacctcat tggaagtgtg cacttctttt tcatcctcac cctcattgtg     300
ctgatcatca cctatcttta ctggcagatc acagagggaa ggaagattat gataaggctg     360
ctccatgagc agatcattaa tgagggcaaa gataaaatgt tcctgataga aaaattgatc     420
aagctgcagg atatggagaa gaaagcaaac cccagctcac ttgttctgga aaggagagag     480
gtggagcaac aaggcttttt gcatttgggg gaacatgatg gcagtcttga cttgcgatct     540
agaagatcag ttcaagaagg taatccaagg gcctgatgac tcttttggta accagacacc     600
aatcaaataa ggggaggaga tgaaaatgga atgatttctt ccatgccacc tgtgccttta     660
ggaactgccc agaagaaaat ccaaggcttt agccaggagc ggaaactgac taccatgtaa     720
ttatcaaagt aaaattgggc attccatgct atttttaata cctggattgc tgatttttca     780
agacaaaata cttggggttt tccaataaag attgttgtaa tattgaaatg agcctacaaa     840
aacctaggaa gagataacta gggaataatg tatattatct tcaagaaatg tgtgcaggaa     900
tgattggttc ttagaaatct ctcctgccag acttcccaga cctggcaaag gtttagaaac     960
tgttgctaag aaaagtggtc catcctgaat aaacatgtaa tactccagca gggatatgaa    1020
```

gcctctgaat tgtagaacct gcatttattt gtgactttga actaaagaca tcccccatgt 1080
cccaaaggtg gaatacaacc agaggtctca tctctgaact ttcttgcgta ctgattacat 1140
gagtctttgg agtcggggat ggaggaggtt ctgcccctgt gaggtgttat acatgaccat 1200
caaagtccta cgtcaagct 1219

<210> 8
<211> 191
<212> PRT
<213> Artificial Sequence

<400> 8

```
Leu Met Met Asn Phe Gln Pro Pro Ser Lys Ala Trp Arg Ala Ser Gln
 1               5                  10                  15
Met Met Thr Phe Phe Ile Phe Leu Leu Phe Phe Pro Ser Phe Thr Gly
            20                  25                  30
Val Leu Cys Thr Leu Ala Ile Thr Ile Trp Arg Leu Lys Pro Ser Ala
        35                  40                  45
Asp Cys Gly Pro Phe Arg Gly Leu Pro Leu Phe Ile His Ser Ile Tyr
    50                  55                  60
Ser Trp Ile Asp Thr Leu Ser Thr Arg Pro Gly Tyr Leu Trp Val Val
65                  70                  75                  80
Trp Ile Tyr Arg Asn Leu Ile Gly Ser Val His Phe Phe Phe Ile Leu
                85                  90                  95
Thr Leu Ile Val Leu Ile Ile Thr Tyr Leu Tyr Trp Gln Ile Thr Glu
            100                 105                 110
Gly Arg Lys Ile Met Ile Arg Leu Leu His Glu Gln Ile Ile Asn Glu
        115                 120                 125
Gly Lys Asp Lys Met Phe Leu Ile Glu Lys Leu Ile Lys Leu Gln Asp
    130                 135                 140
Met Glu Lys Lys Ala Asn Pro Ser Ser Leu Val Leu Glu Arg Arg Glu
145                 150                 155                 160
Val Glu Gln Gln Gly Phe Leu His Leu Gly Glu His Asp Gly Ser Leu
                165                 170                 175
Asp Leu Arg Ser Arg Arg Ser Val Gln Glu Gly Asn Pro Arg Ala
            180                 185                 190
```

<210> 9
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 9

```
Ile Thr Glu Gly Arg Lys Ile Met Ile Arg
 1               5                  10
```

<210> 10
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 10

```
Leu Leu Met Asp Phe Val Phe Ser Leu
 1               5
```

<210> 11
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 11

```
Phe Leu Leu Phe Phe Pro Ser Phe Thr
 1               5
```

<210> 12
<211> 9
<212> PRT

<213> Artificial Sequence

<400> 12
Gln Met Met Thr Phe Phe Ile Phe Leu
1 5

<210> 13
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 13
Met Met Thr Phe Phe Ile Phe Leu Leu
1 5

<210> 14
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 14
Phe Leu Ile Glu Lys Leu Ile Lys Leu
1 5

<210> 15
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 15
Val Leu Leu Ile Arg Asn Ile Phe Leu
1 5

<210> 16
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 16
Leu Val Trp Ile Gly Ile Phe Phe Cys
1 5

<210> 17
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 17
Thr Leu Ala Ile Thr Ile Trp Arg Leu
1 5

<210> 18
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 18
Leu Ile Phe Cys Trp Asp Phe Thr Val
1 5

<210> 19
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 19

Phe Leu Gly Glu Phe Leu Arg Arg Ile
1 5

<210> 20
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 20
Leu Leu Leu Pro Phe Val Val Ser Cys
1 5

<210> 21
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 21
Leu Leu Thr Arg Phe Ser Ala Tyr Met
1 5

<210> 22
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 22
Lys Leu Ile Phe Cys Trp Asp Phe Thr
1 5

<210> 23
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 23
Leu Leu Leu Met Asp Phe Val Phe Ser Leu
1 5 10

<210> 24
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 24
Leu Leu Met Asp Phe Val Phe Ser Leu Val
1 5 10

<210> 25
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 25
Gln Met Met Thr Phe Phe Ile Phe Leu Leu
1 5 10

<210> 26
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 26
Tyr Leu Ala Glu Tyr Asn Leu Glu Phe Leu
1 5 10

<210> 27
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 27
Lys Leu Il Phe Cys Trp Asp Phe Thr Val
1 5 10

<210> 28
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 28
Leu Leu Phe Phe Pro Ser Phe Thr Gly Val
1 5 10

<210> 29
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 29
Gln Leu Leu Thr Arg Phe Ser Ala Tyr Met
1 5 10

<210> 30
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 30
Thr Leu Val Trp Ile Gly Ile Phe Phe Cys
1 5 10

<210> 31
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 31
Ser Gln Met Met Thr Phe Phe Ile Phe Leu
1 5 10

<210> 32
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 32
Val Leu Leu Leu Pro Phe Val Val Ser Cys
1 5 10

<210> 33
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 33
Ile Leu Cys Tyr Tyr Trp Leu Asn Thr Val
1 5 10

<210> 34
<211> 10
<212> PRT

<213> Artificial Sequence

<400> 34
Thr Leu Ile Val Leu Ile Ile Thr Tyr Leu
1 5 10

<210> 35
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 35
Tyr Val Leu Leu Ile Arg Asn Ile Phe Leu
1 5 10

<210> 36
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 36
Phe Val Phe Ser Leu Val Asn Ser Phe Leu
1 5 10

<210> 37
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 37
Leu Leu Leu Pro Phe Val Val Ser Cys Ile
1 5 10

<210> 38
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 38
Leu Ile Gly Ser Val His Phe Phe Phe Ile
1 5 10

<210> 39
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 39
Tyr Glu Met Pro Arg His Glu Val Tyr Val
1 5 10

<210> 40
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 40
Leu Leu Thr Arg Phe Ser Ala Tyr Met Val
1 5 10

<210> 41
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 41

Ile Tyr S r Gly Gly Ile Thr Lys Leu
1 5

<210> 42
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 42
Val Tyr Tyr Leu Ala Glu Tyr Asn Leu
1 5

<210> 43
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 43
Tyr Tyr Trp Leu Asn Thr Val Ala Leu
1 5

<210> 44
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 44
Ile Tyr Arg Leu Leu Leu Met Asp Phe
1 5

<210> 45
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 45
Val Tyr Val Leu Leu Ile Arg Asn Ile Phe
1 5 10

<210> 46
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 46
Cys Tyr Tyr Trp Leu Asn Thr Val Ala Leu
1 5 10

<210> 47
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 47
Tyr Tyr Leu Ala Glu Tyr Asn Leu Glu Phe
1 5 10

<210> 48
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 48
Met Leu Phe Ile Met Phe Tyr Ser Lys
1 5

<210> 49
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 49
Leu Leu Ile Arg Asn Ile Phe Leu Lys
1 5

<210> 50
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 50
Gly Leu Gln Glu Phe Asp Ile Ala Arg
1 5

<210> 51
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 51
Lys Met Phe Leu Ile Glu Lys Leu Ile Lys
1 5 10

<210> 52
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 52
Ile Met Leu Phe Ile Met Phe Tyr Ser Lys
1 5 10

<210> 53
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 53
Lys Leu Ile Lys Leu Gln Asp Met Glu Lys
1 5 10

<210> 54
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 54
Val Leu Leu Ile Arg Asn Ile Phe Leu Lys
1 5 10

<210> 55
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 55
Met Pro Arg His Glu Val Tyr Val Leu
1 5

<210> 56
<211> 10
<212> PRT

<213> Artificial Sequence

<400> 56
Met Pro Arg His Glu Val Tyr Val Leu Leu
1 5 10

<210> 57
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 57
Tyr Glu Met Pro Arg His Glu Val Tyr
1 5

<210> 58
<211> 10
<212> PRT
<213> Artificial Sequence

<400> 58
Trp Glu Thr Leu Ile Gly Gln Asp Ile Tyr
1 5 10

<210> 59
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 59
Val His Phe Phe Phe Ile Leu Thr Leu
1 5

<210> 60
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 60
Leu Arg Arg Ile Ile Gly Met Gln Leu
1 5

<210> 61
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 61
Leu Leu Met Asp Phe Val Phe Ser Leu
1 5

<210> 62
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 62
Met Gln Leu Ile Thr Ser Leu Gly Leu
1 5

<210> 63
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 63

Phe Val Phe Ser Leu Val Asn Ser Phe
1 5

<210> 64
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 64
Phe Ile Leu Thr Leu Ile Val Leu Ile
1 5

<210> 65
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 65
Trp Gln Ile Thr Glu Gly Arg Lys Ile
1 5

<210> 66
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 66
Met Phe Tyr Ser Lys Asn Ile Ser Leu
1 5

<210> 67
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 67
Phe Ile Met Phe Tyr Ser Lys Asn Ile
1 5

<210> 68
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 68
Trp Arg Ala Ser Gln Met Met Thr Phe
1 5

<210> 69
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 69
Phe Phe Phe Ile Leu Thr Leu Ile Val
1 5

<210> 70
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 70
Phe Arg Leu Val Glu Arg Tyr Glu Met
1 5

<210> 71
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 71
Phe Phe Ile Leu Thr Leu Ile Val Leu
1 5

<210> 72
<211> 9
<212> PRT
<213> Artificial Sequence

<400> 72
Tyr Ala Gln Thr Leu Val Trp Ile Ala
1 5